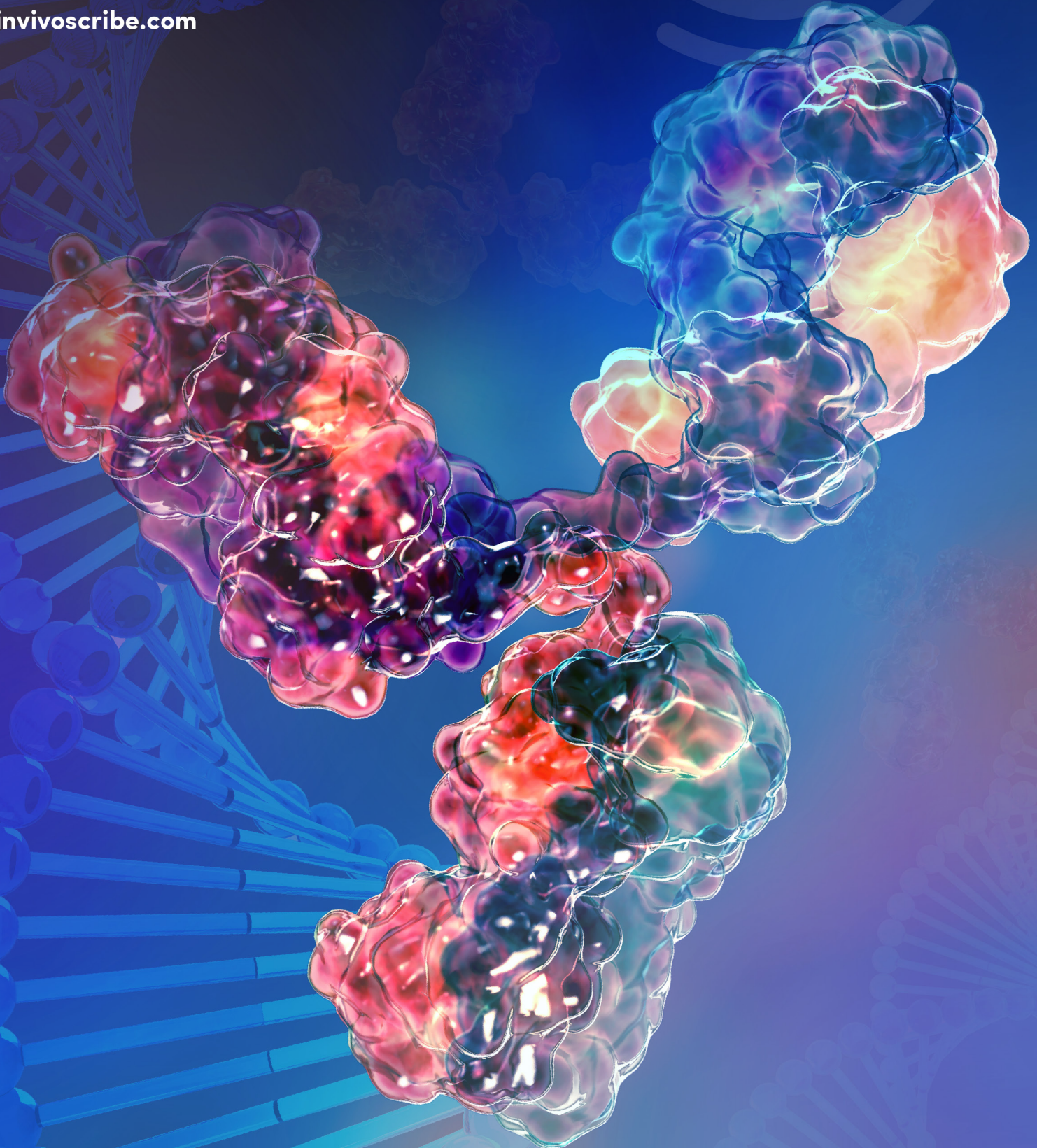



 **invivoscribe**[®]

2022 Product Catalog

invivoscribe.com





Invivoscribe, Inc. is a technology-driven international company focused on precision diagnostics with best-in-class products and services that optimize patient care and accelerate drug approvals worldwide.

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Improving Lives with Precision Diagnostics[®]



[invivoscribe.com](https://www.invivoscribe.com)

 **invivoscribe**

Dear Colleagues,

I am pleased to introduce Invivoscribe's 2022 Product Catalog. As in years past, I am taking this opportunity to highlight a few of our company's many accomplishments in 2021 and identify a few of the many projects we will address in the coming year.

To highlight just a few of the accomplishments our team members achieved in 2021:

Invivoscribe evolved its Quality Management System (QMS) to meet the IVDR requirements and submitted the first class C CDx package for IVDR review to our notified body, helping cement our role as a leader in ensuring state of the art targeted therapies are supported in the EU.

Invivoscribe's software and bioinformatics teams finalized development and launched updated MRD Software that allows simultaneous tracking of up to five separate biomarkers using our LymphoTrack® assays.

Our bioinformatics team, working with our flow team and under direction of our chief medical officer, developed and presented new machine learning algorithms to facilitate and complement manual interpretation of flow cytometry data by hematopathologists. Initial data from the algorithms were presented in a supplemental issue of *Blood* and in a poster at ASH.

We began work on moving bioinformatics software pipeline and reporting features to the cloud. When complete, we expect our portal will revolutionize and expand our commercial platform, providing access to bioinformatics pipeline and software for data analysis, interpretation for pharma partners and customers worldwide. In addition, we have started licensing an enterprise version of Invivoscribe's LymphoTrack® Software to streamline data management at key cancer centers.

Invivoscribe expanded R&D, bioinformatics and software teams in San Diego in order to develop a variety of assays and furthered our collaboration with a number of clinical pharmaceutical partners using LymphoTrack *IGH* and *TCR* assays to support minimal residual disease (MRD) detection in international clinical studies of a wide range of hematologic malignancies, including multiple myeloma (MM), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), peripheral T-cell lymphomas (PTCL). A number of scientific papers and posters were also published or presented by clinical cancer centers using LymphoTrack® MRD assays. We had 34 peer-reviewed scientific papers published by global customers conducted using Invivoscribe products and participated in 13 international conferences.

LabPMM, our international clinical laboratory arm, expanded capabilities across sites via obtaining CAP/CLIA listing for flow in the US, validating additional flow lab and molecular testing capabilities in China, moving our EU lab to a larger more sophisticated facility in Munich, and submitting for ISO 15189 accreditation in Japan. This year we will be offering an even more complete menu of PCR capillary assays, NGS tests, gene panels, bioinformatics, and multiparameter flow cytometry screening and MRD panels to support clinical and pharma partners in the LabPMM laboratories around the world.

Our flow cytometry team developed screening assays for hematologic malignancies. The CLIA-accredited 10-color Hematolymphoid Screening Panel provides a comprehensive approach to evaluate bone marrow and peripheral blood for the presence or absence of hematolymphoid malignancies. The team also developed and launched a 12-color flow MRD test for AML that tracks 21 biomarkers. These panels are currently being run in San Diego, but reagent set and software validations with the addition of cGMP manufacturing ensure that we can make these assays available in China and other LabPMM sites in the near future.

We are developing a pan cancer gene panel, and 2nd generation MyAML® and MyMRD® gene panels with more genes and better sensitivity to identify and monitor variants such as *TP53* in solid tumors, myeloid malignancies, including acute myeloid leukemia (AML), myeloproliferative neoplasms (MPN), and myelodysplastic syndromes (MDS).

Some key Invivoscribe and LabPMM milestones that will be met this year include:

The team will be submitting for CAP accreditation of our Shanghai laboratory; finishing our ISO 15189 accreditation for our lab in Japan; earning our IVDR QMS accreditation at our San Diego headquarters; receiving LeukoStrat CDx *FLT3* Mutation Assay approval through the new IVDR system in the EU; and generating IVDR validation packages for multiple capillary and NGS assays in the queue for submission to our notified body. Additionally, we will be finalizing development and release an NGS-based B-cell MRD Assay, an MRD Myeloid Assay, and machine learning algorithms to assist in streamlining flow analyses in our clinical laboratories worldwide.

Finally, I want to acknowledge that our growth and progress over these past 27 years would not be possible without your feedback and support. We look forward to continued interaction with research and clinical colleagues to ensure we can continue to offer you the best products for decades to come.

We wish you, your colleagues, and your families a safe, joyful, productive, and successful 2022.

Sincerely Yours,



Jeffrey Edward Miller, Ph.D.

*Founder, Chief Scientific Officer,
Chief Executive Officer & Chairman*

A handwritten signature in black ink, appearing to read 'Jeff Miller', written in a cursive style.



Board of Directors



Hardwick Simmons, MBA

Hardwick 'Wick' Simmons retired as the Chairman and CEO of The Nasdaq Stock Market, Inc. in May of 2003. Prior to Nasdaq, he served as President and CEO of Prudential Securities Inc., a major investment management and securities brokerage firm. Simmons is a former chairman of the Securities Industry Association, a former director of the Chicago Board Options Exchange and a former president of the New York Bond Club. He is currently a director of Lionsgate Entertainment Corp., president of Stonetex Oil Corp., and a trustee of Woods Hole Oceanographic Institution. He is a graduate of Harvard College and Harvard Business School and served in the U.S. Marine Corps Reserve from 1960 until 1966.



Mitchell Kronenberg, Ph.D.

Mitchell Kronenberg received a B.A. from Columbia University, a Ph.D. from the California Institute of Technology, and served on the faculty of the UCLA School of Medicine from 1986-1997. He joined the La Jolla Institute for Immunology in 1997, and has been the President there since 2003. The Institute has grown in accomplishment and reputation under his leadership. Dr. Kronenberg's research interests include natural killer T cells, other innate lymphocytes such as MAIT cells and ILC, regulation of mucosal immunology and the microbiome and pathogenesis of inflammatory bowel disease. He has co-authored more than 350 publications, and is a fellow of the American Association for the Advancement of Science (AAAS), a Distinguished Fellow of the American Association of Immunologists, recipient of an NIH MERIT award and is an Institute for Scientific Information (ISI) Highly Cited Scientist. In 2016, he was named the most admired CEO (large nonprofit organization category) by the San Diego Business Journal. He is an advisor to a number of organizations including service as a member of the Board of Scientific Counselors for Basic Science, National Cancer Institute and he has been involved with Invivoscribe since its founding.



Stephen Wilson, Ph.D.

Dr. Wilson is a biomedical research executive with more than two decades of experience in basic and translational immunology. He received his B.S.A. and Ph.D. from the University of Arizona, and serves as the Executive Vice President and Chief Operating Officer of the La Jolla Institute for Immunology.



Jeffrey Edward Miller, Ph.D.

Dr. Miller is a scientist, inventor, and entrepreneur focused on Improving Lives with Precision Diagnostics® – coupling drug trials and therapeutic treatment regimens with optimized clinically-actionable diagnostic methods in order to select the correct patients and then monitor and track their response throughout the course of their disease. He received his undergraduate degree in Biochemistry from UCLA and a combined Ph.D. in Biochemistry & Molecular Biology from UCSB. Prior to starting Invivoscribe, Dr. Miller had more than twenty years of combined experience in protein biochemistry, cellular and molecular immunology, cardiac physiology, virology, and molecular biology, experience he had developed working in laboratories at the Medical School, Department of Chemistry, Molecular Biology Institute at UCLA, while earning his Ph.D. at UCSB, and as a postdoctoral scientist at Applied Molecular Evolution. He also spent several years at Quest Diagnostics Nichols Institute, setting up the molecular oncology laboratory and developing and launching PCR-based molecular assays for infectious disease and hematopathology.



James Isaacs, Jr., JD.

James B. Isaacs, Jr. has practiced law since 1983. He currently serves as Licensing and Contracts Counsel at Invivoscribe. Jim attended Stanford University and Yale Law School, then began his career at the Los Angeles law firm of O'Melveny & Myers. As a trial lawyer and later in-house counsel with a focus on intellectual property disputes, Jim has successfully defended a myriad of businesses and individuals; in plaintiffs' actions he has obtained and collected multi-million dollar judgments in the United States and abroad. As a businessman and co-founder of Invivoscribe, Jim has been active in the legal and commercial affairs of the company since 1995.



Gary Clouse, JD.

Gary Clouse has practiced as a litigator and business attorney in Southern California for more than three decades. He currently serves as Corporate Secretary and Legal Counsel for Special Projects at Invivoscribe. Clouse is a graduate of Indiana State University and Northwestern University School of Law. Following law school, he clerked for the federal Seventh Circuit Court of Appeals in Chicago. He began his legal practice at the law firm O'Melveny & Myers in Los Angeles. Clouse is one of the founders of invivoscribe.

Executive Leadership Team



Jeffrey Edward Miller, Ph.D.

Founder, Chief Scientific Officer, & Chief Executive Officer

Dr. Miller is a scientist, inventor, and entrepreneur focused on Improving Lives with Precision Diagnostics® – coupling drug trials and therapeutic treatment regimens with optimized clinically-actionable diagnostic methods in order to select the correct patients and then monitor and track their response throughout the course of their disease. He received his undergraduate degree in Biochemistry from UCLA and a combined Ph.D. in Biochemistry & Molecular Biology from UCSB. Prior to starting Invivoscribe, Dr. Miller had more than twenty years of combined experience in protein biochemistry, cellular and molecular immunology, cardiac physiology, virology, and molecular biology, experience he had developed working in laboratories at the Medical School, Department of Chemistry, Molecular Biology Institute at UCLA, while earning his Ph.D. at UCSB, and as a postdoctoral scientist at Applied Molecular Evolution. He also spent several years at Quest Diagnostics Nichols Institute, setting up the molecular oncology laboratory and developing and launching PCR-based molecular assays for infectious disease and hematopathology.



Meghna Bhatnagar, MBA

Chief Financial Officer

Meghna Bhatnagar joined Invivoscribe in 2010 as Chief Financial Officer. In this role she is responsible for leading the Invivoscribe global finance organization, along with human resources and information technology. Since her arrival, Ms. Bhatnagar has played an integral role in directing all aspects of company strategy, planning and operations. Ms. Bhatnagar has over 20 years of experience building and leading finance and IT teams in global companies. Prior to joining IVS, she served as COO of Radiant Technologies, a technology company focused on providing business management solutions to small and medium sized companies where she was responsible for leadership and development of an entire project delivery team with full P&L responsibility. She played a key role in guiding overall strategy of the company and at the same time provided leadership for operational improvements.



Tony Lialin

Chief Commercial Officer

Tony Lialin joined Invivoscribe in 2021 as Chief Commercial Officer. In this role he is responsible for leading the global commercial organization. Mr. Lialin has more than two decades of experience in growing companies in the life sciences industry having held positions in clinical diagnostic, bioinformatics software, instrumentation and reagent companies. He has successfully scaled commercial operations at three start-ups resulting in those companies being purchased by large public companies. Mr. Lialin comes to IVS from Loop Genomics, an NGS long-read sequencing company where he started their global commercial operations growing their business to several million dollars in revenue per year in just three years. From 2014 through June 2018, Mr. Lialin managed \$400M in portfolio business at Illumina working closely with customers in the DTC, Oncology and Postnatal testing spaces. He also served as a director at Agilent Technologies, where he managed the emerging market, field application scientist, and technical support and field service engineering teams. As a six-sigma green belt he values process, service as a differentiator and both internal and external customers. Mr. Lialin earned a BA in Molecular Cellular and Developmental Biology from the University of California at Santa Cruz.



Dr. Bradley Patay, M.D.

Chief Medical Officer

Dr. Patay is dedicated to improving health by integrating genomic knowledge into medical care. He has authored numerous articles in this field, presented at multiple conferences and has been featured in Bloomberg Business Week. He has been head of the internal medicine section at Scripps Torrey Pines, worked as an Assistant Professor at STSI, and has been a founding member of the Board, and Vice President of the College of Genomic Medicine, which was established in 2010 to educate physicians and other health care professionals about genomic medicine. His diverse clinical experience prior to joining Scripps Clinic in 2005 includes four years as an internist and pediatrician at Neighborhood Healthcare, a private, nonprofit community healthcare practice, and at Palomar Hospital. At these institutions, Brad cared for a wide range of patients, from neonates to the elderly, in both intensive care and the general wards. Through his service on several committees, he helped improve health care institutions' systems.



Tania Obranovich, BSc (Hons) PhD LLB

Chief IP and Legal Strategy Officer

Dr. Tania Obranovich has more than 25 years' experience developing and managing global IP portfolios in the biotechnology sector, in particular in relation to molecular, cellular and genomic technologies as they relate to diagnostics and therapeutics. She has acquired this experience both through private practice, having spent more than a decade as a partner in a top tier IP firm, and as legal counsel for a US based diagnostics company where she ran patent and trade mark prosecution and drafting, in addition to overseeing all legal, compliance and corporate governance matters. Tania has practiced throughout her career as both a lawyer and patent attorney. Prior to these roles she completed a PhD and post-doctoral fellowship in immunology. In addition to her legal practice, Tania has lectured extensively in patent law at The University of Melbourne and Monash University and also speaks widely in relation to IP issues. She was heavily involved with navigating the Australian and US legal and policy issues that arose as a result of the Myriad gene patent litigation in both these jurisdictions. Tania received a Federal Government appointment to the Professional Standards Board for Patent and TM Attorneys in 2012 and has held several other Board positions. Tania has served as Chief IP and Legal Strategy Officer to Invivoscribe since 2019.



Dr. Meindert Niemeijer, Ph.D., M.Sc.

Chief Information Officer

Dr. Niemeijer came to IVS from Digital Diagnostics where he led the teams that designed, built, documented and put into production the first autonomous diagnostic Artificial Intelligence (AI), cleared by the FDA. He is an experienced technology leader and deep learning expert passionate about developing AI based medical devices. During his career, he has built a deep expertise in medical device (software) development processes and the way in which these interact with quality management and other regulatory requirements. He loves building and developing technology, processes and teams. Dr. Niemeijer obtained a Ph.D. in Medical Image Analysis and Machine Learning from Utrecht University in the Netherlands. In addition, he has an M.Sc. in Medical Computer Science, also from Utrecht University.

Leadership Team Continued



Paul McMullin, MIM, MBA

Head of Global Sales and Marketing

Paul McMullin joined Invivoscribe in 2018 as the Head of Global Sales & Marketing. In this role, he leads Invivoscribe's worldwide Sales and Marketing efforts with focus on both Diagnostic and Companion Diagnostics (CDx) Products. These Products are sold in over 100 Countries via a Direct Sales Organization as well as Exclusive Distributors. Mr. McMullin has over 35 years of experience working in the Medical Diagnostics Business. Most of that time has been in the molecular and biotechnology fields. His experience includes numerous positions in Sales and Marketing, establishing Direct Sales, Service and Support companies in Europe and Australia, and managing Distributor Sales in over 55 countries worldwide. He has directly managed employees in Japan, China and most major EU countries.



Jason Gerhold

Global Director, Regulatory Affairs/Quality Assurance

Jason Gerhold joined Invivoscribe in 2012 and serves as the Global Director of Regulatory, Quality, and Clinical Affairs focusing on developing high quality diagnostics that meet both international pathology needs and support pharma partner's drug development programs. He directly manages employees in the US, Japan, and China, and is responsible for worldwide compliance to relevant laws, regulations, and standards. Mr. Gerhold has over 20 years of experience in the biotech industry with biologic therapeutics and diagnostics, including process development, regulatory, quality, and clinical roles. Mr. Gerhold has worked in small, medium and large companies; successfully registering IVD and CDx products in several international markets, developing, improving, and maintaining quality management systems while serving as the management representative to regulatory authorities, and leading clinical teams managing international registrational studies. He is known for building strong rapport with regulators and working well with research and pharma teams resulting in highly successful partnerships. He has completed trainings and/or accreditations for ISO 13485 Lead Auditor, Six Sigma Greenbelt, Clinical Trials Administration, Project Management, and CTD writing.



Jordan Thornes

Global Director, Clinical Labs

Jordan Thornes joined Invivoscribe in 2004 and currently serves as the Global Director of Clinical Labs. In this role, he is responsible for leading Invivoscribe's global network of clinical labs, The Laboratory for Personalized Molecular Medicine®, (LabPMM®). There are currently four such LabPMM facilities which are strategically located globally in San Diego, USA; Hallbergmoos (Munich), Germany; Shanghai, China and Kawasaki (Tokyo), Japan. LabPMM aids patients and pharmaceutical companies worldwide with globally standardized and clinically-actionable diagnostic methods. LabPMM utilizes proprietary biomarkers to select the correct patients and monitor and track their critically important response throughout the course of their disease.



Andreia de Albuquerque, Ph.D.

Director of Global Business Development

Andreia de Albuquerque joined Invivoscribe in 2015 and currently serves as the Director of Business Development. In this role, she is responsible for leading Invivoscribe's global partnerships with Pharma and Biotech's, focusing on companion diagnostics development, custom assay development, clinical trial testing, corporate partnerships and other strategic relationships. Prior to joining Invivoscribe, Dr. de Albuquerque has held roles in precision molecular diagnostics, focusing on development, implementation and validation of PCR and NGS assays for diagnostic and research purposes. Her work has resulted in several peer-reviewed publications and participations in international conferences. During this time, she was also responsible for overseeing laboratory diagnostic teams and operations. Her extensive expertise in precision diagnostics and understanding of assay development and clinical testing, paved the way into Business Development, where she focus on accelerating drug approvals, by building successful partnerships, providing technical expertise and working closely with biomarker leaders, sample operations, clinical science and regulatory to implement CDx, IVD and R&D assays in clinical trials. Dr. de Albuquerque earned her degree in Biology from the University of Coimbra (Portugal) and a combined Ph.D. in Oncology and Molecular Biology from the University of Dresden (Germany).



Ying Huang, Ph.D.

Senior Director of Product Development

Dr. Ying Huang joined Invivoscribe in 2010 and currently serves as the Sr. Director of Product Development. In this role, she is responsible for leading Invivoscribe's product development activities to address unmet clinical needs in the fields of molecular diagnostic and precision medicine. Dr. Huang has over 25 years of research and industry experience with a broad knowledge in technology and assay development. She is passionate about developing novel methods using cutting edge technologies to improve lives with precision diagnostics. She has successfully led development teams through entire product development life cycle, from feasibility to RUO, CLIA and IVD grade product launches and post market support under respective regulatory standards. Prior to Invivoscribe, Dr. Huang worked at Illumina, ACEA Biosciences, Nanogen and MD Anderson Cancer Center. In those roles, she developed a variety of techniques ranging from isolating CTC using microfluidic devices to detecting biowarfare agents using electronic microarray. Dr. Huang was the PI for research grants from DARPA and NIH. She is the co-author of more than 60 publications and meeting abstracts and is the co-inventor of 9 patents. Dr. Huang received her Ph.D. in Electrical Engineering from Bangor University, UK and B.E. in Electronic Engineering from Xidian University, China.

Best-in-Class Assays & Reagents



invivoscribe.com/products/

Invivoscribe provides a full range of standardized CE-marked in vitro diagnostic cGMP products for hematology-oncology, as well as RUO assays, analyte specific reagents (ASRs), and DNA & RNA controls.



Best-in-Class Assays & Reagents

Invivoscribe provides a full range of standardized CE-marked *in vitro* diagnostic cGMP products for hematology-oncology, as well as RUO assays, analyte specific reagents (ASRs), and DNA & RNA controls.

Next-Generation Sequencing

NGS is a powerful, high-throughput DNA sequencing technology that allows for massively parallel sequencing of millions of DNA fragments in a single sequencing run. NGS is revolutionizing modern science and healthcare.

ABI Fluorescence Detection and Gel Detection

We exclusively offer a comprehensive selection of PCR-based assays for ABI fluorescence detection and gel detection, including targeted *FLT3* ITD and TKD mutation assays, B- and T-cell clonality assays (based on EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936), and translocation assays.

Controls & Reagents

Invivoscribe offers an extensive range of General Purpose Reagents (GPRs) and Research Use Only (RUO) nucleic acid controls.

Companion Diagnostics (CDx)

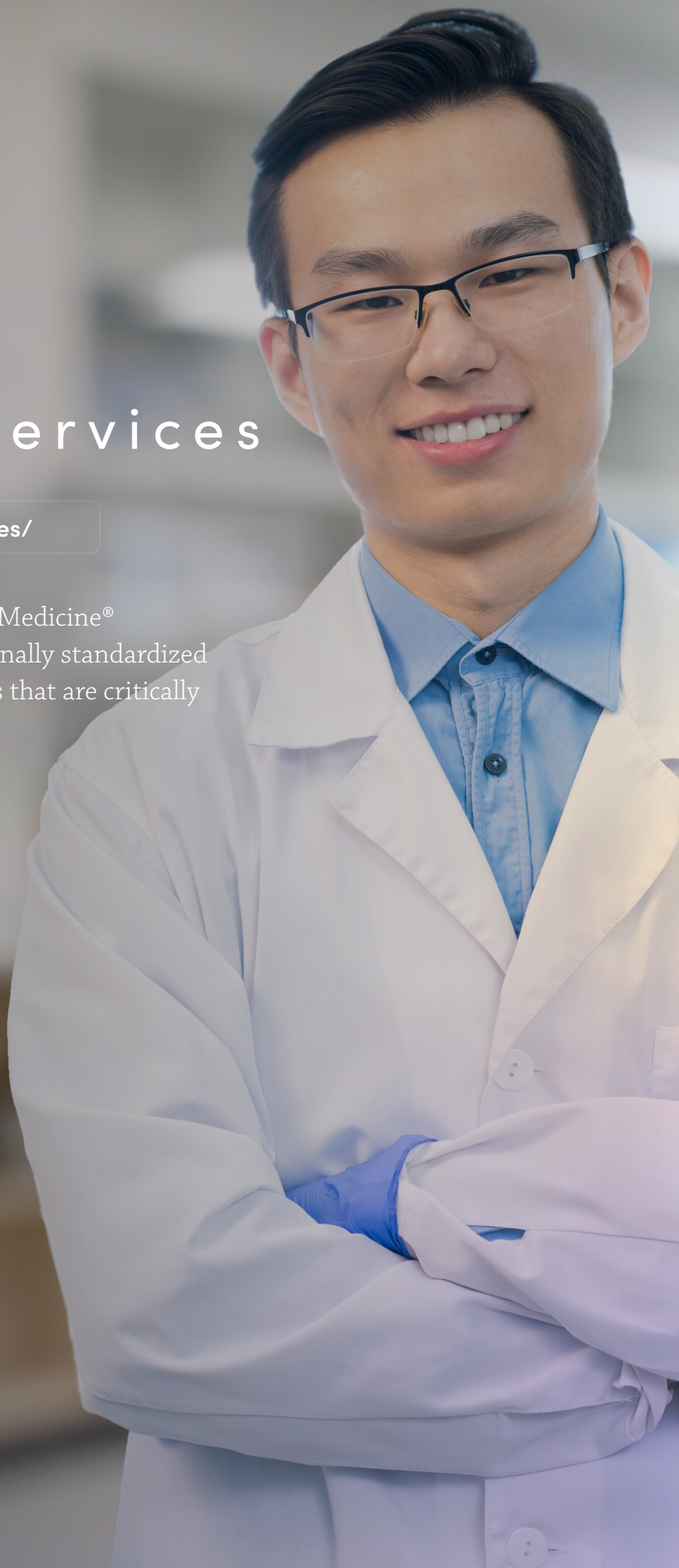
Invivoscribe is a Comprehensive Partner for Companion Diagnostic Development.

LabPMM[®] Clinical Lab Services



invivoscribe.com/clinical-lab-services/

The Laboratory for Personalized Molecular Medicine[®] (LabPMM[®]) at Invivoscribe offer internationally standardized testing of novel and proprietary biomarkers that are critically important for patient care.



LabPMM® Clinical Lab Services

The Laboratory for Personalized Molecular Medicine® (LabPMM®) at Invivoscribe offer internationally standardized testing of novel and proprietary biomarkers that are critically important for patient care.

NGS Cancer Panels

Cytogenetic identification of chromosome abnormalities has become essential for the clinical management of patients with leukemia and is currently used to help categorize patients into risk groups.

MRD NGS Tests

LabPMM's B- and T-cell MRD tests are NGS-based assays that can be used to detect clonal gene rearrangements identified at diagnosis within virtually all of the antigen receptor loci for both B- and T-cells. LabPMM's *NPM1* MRD and *FLT3* MRD tests are NGS-based assays that can be used to detect targeted mutations identified at diagnosis.

Targeted Genes

An *NPM1* assay is offered to detect targeted mutations. *NPM1* and *FLT3* mutations can be used for risk stratification and prognosis of AML.

Clonality Tests

The unique process of genetic rearrangements in the immunoglobulin (Ig) and T-cell receptor (TCR) gene loci during immune cell development and maturation generates a vast pool of genetically distinct cells. Hematologic malignancies are characterized by the reduced population diversity of these gene loci originating from the proliferative transformation of an individual lymphoid cell. The associated cellular population typically shares one or more cell-specific or "clonal" antigen-receptor gene rearrangements. The detection of these clonal cells provides the basis for clonality assessment in leukemia, lymphoma, and hematologic disease diagnosis.

Multiparametric Flow Cytometry

LabPMM offers standardized and sensitive multiparametric flow cytometry (MFC) assays that allow up to 12 parameters to be simultaneously analyzed on a single cell. Our advanced instrumentation and greater selection of fluorochromes allows us to collect more clinically relevant information with smaller sample sizes thus providing more information for the patient. These highly specific and sensitive techniques are utilized in screening/diagnostic work up of leukemia/lymphomas and rare event analysis such as minimal/measurable disease (MRD) testing in various disease states such as Acute myeloid leukemia, Multiple myeloma and Chronic lymphocytic leukemia.

Companion Diagnostics (CDx) Tests

CDx enable the identification of biomarkers that indicate the patients most likely to benefit (or alternately not benefit) from specific or combinatorial targeted therapies. Their utilization enhances patient care and the potential for improved outcomes. The fms related tyrosine kinase 3 (*FLT3*) is one of the most commonly mutated genes in acute myeloid leukemia (AML), occurring in approximately 30% of patients at the time of diagnosis.¹ Although generally associated with normal cytogenetics where patients have standard risk of relapse, *FLT3* mutations have also been identified in sub-groups of patients with chromosomal abnormalities that are associated with high risk of disease relapse.²⁻³ The LeukoStrat CDx *FLT3* Mutation Assay is a globally standardized test validated for detection of genetic mutation in the *FLT3* gene which is one of the most important driver mutations in AML.

Custom Assays

In response to the FDA announcing its intention to dramatically expand its regulatory oversight of laboratory developed tests (LDTs), Invivoscribe is partnering with laboratories worldwide to help facilitate the conversion of LDTs into FDA-cleared assays, as we know the barriers to bringing new assays online are often the availability of resources and the cost of validation.

References | 1. Acute Myeloid Leukemia, *Clinical Practice Guidelines in Oncology*, (v.2.2014) National Comprehensive Cancer Network. | 2. Lowenberg, B. et al. (1999) "Acute myeloid leukemia." *N Engl J Med* 341(14):1051-62. | 3. Thiede, C. et al. (2002) "Analysis of *FLT3*-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB and identification of subgroups with poor prognosis." *Blood* 99(12): 4326-35.

Streamlined CDx Partnerships



invivoscribe.com/partnerships/

Companion Diagnostics represents a critical milestone in precision medicine and plays a pivotal role in the validation of targeted therapies. Traditional CDx development models involve multiple partners adding complexity and increasing risk which could affect drug approvals.



Streamlined CDx Partnerships

Our Streamlined CDx™ program has demonstrated value, accelerating the international approvals of multiple new targeted therapies for multiple pharmaceutical companies.

Product Development

Design Controls

» All biomarker assays & software developed under design controls.

Bioinformatics Software

» Comprehensive LymphoTrack® Dx clonality with bioinformatics software.

Identify & Track

» Multiple NGS gene panels that identify and track clinically-actionable biomarkers.

Custom Development

» Custom biomarker assay development.

Complementary MRD Assays

» Complementary MRD assays for all biomarkers – potential for surrogate endpoints per agency inputs.

Manufacturing

cGMP Compliant

» US FDA/CDRH registered, EN ISO 13485:2016 certified manufacturing facility based in San Diego.

CE-marked IVDs & CDx Manufacturing

» PMA companion diagnostics (CDx) for US and Japan, inclusion of CDx in ARTG Australia and >50 CE-IVDs (NGS assays include bioinformatics software).

Assay Development

» IUO & RUO assays, CE-marked IVDs, & CDx.

Controls & Reagents

» DNA / RNA controls, ASRs, GPRs, MRD controls & proficiency panels.

Clinical Lab Services

Clinical Lab Experience

» A dozen years of clinical reference lab experience.

Internationally Standardized

» Internationally standardized CDx, flow cytometry and biomarker testing with internationally accredited labs serving the US, Europe, and Asia.

Comprehensive Panels

» Comprehensive B- and T-cell clonality/MRD assays and NGS MyGene™ panels identify clinically-actionable biomarkers.

MOA Profiling of Novel Compounds

» Using primary AML patient samples – IC50 determination, mRNA analysis, genetic, and phenotypic characterization.

Worldwide Enrollment

» Testing services have supported hundreds of enrollment sites worldwide.

Global Regulatory Expertise

Accredited & Proven

» EN ISO 13485:2016 accredited.

» Experienced staff & proven Quality Management System.

Registered Medical Device Establishment

» Registered Medical Device Establishment with the US FDA, KFDA, Saudi Arabia, and the MHLW/PMDA.

Multiple CDx Approvals

» Multiple CDx approvals supporting various drugs: by the FDA (US), PMDA/MHLW (Japan), and TGA (Australia).

» CDx CE-marked IVD in the EU.

CE-marked IVDs

» More than 50 CE-marked IVDs available in the EU and select ROW markets; >60 tests included in the ARTG in Australia.

Marketing Authorization Holder (MAH)

» Marketing Authorization Holder (MAH) and national reimbursement for CDx in Japan.

Global Distribution Network



invivoscribe.com/company/global-distributors/

A long and proud history of working with scientists, laboratorians and clinicians from around the world. We offer product support in over 160 countries. Our distributor partners are in locations around the globe.

AFRICA

Algeria
Angola
Burkina Faso
Burundi
Cameroon
Chad
Côte d'Ivoire
Egypt
Equatorial Guinea
Eswatini
Ethiopia
Gabon
Ghana
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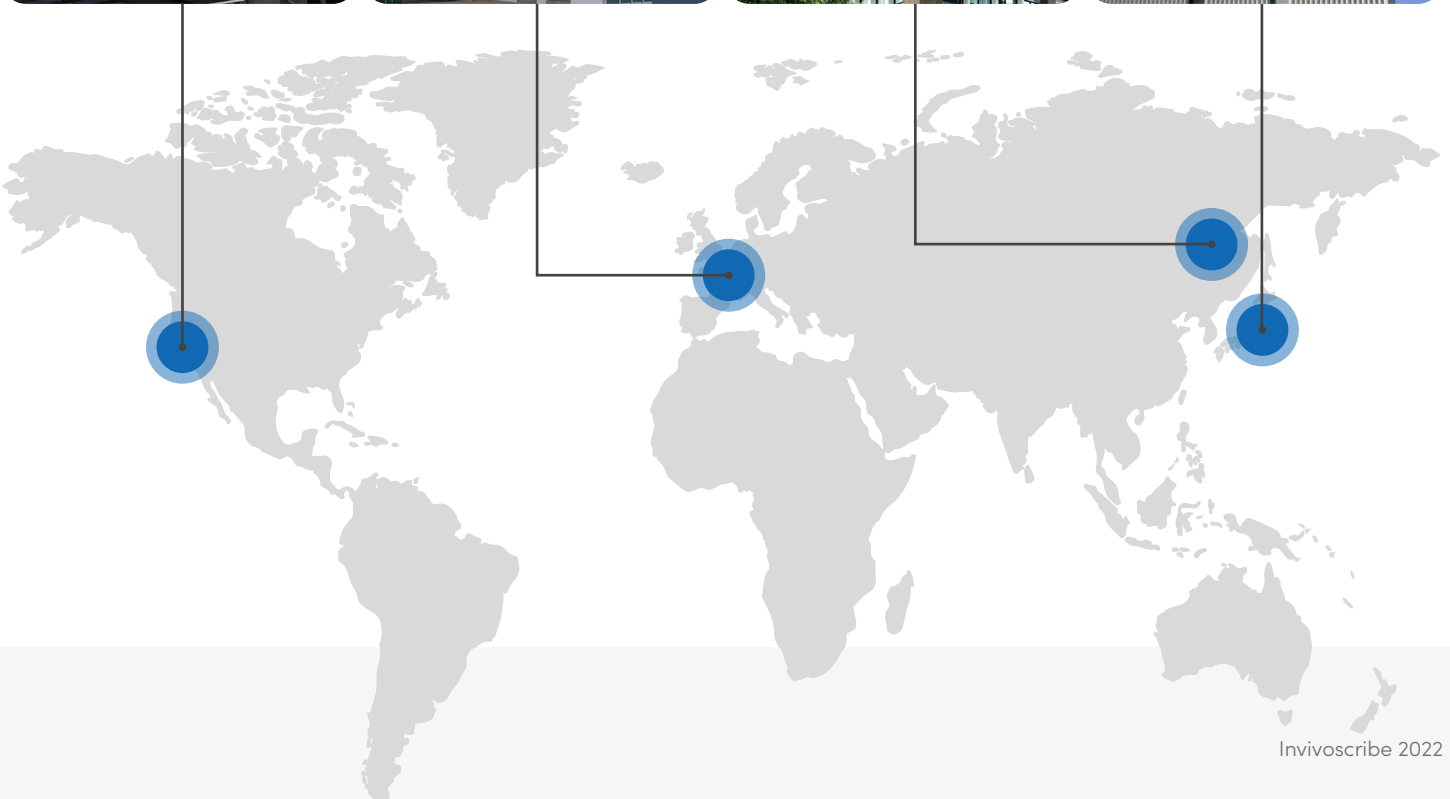
Munich, Germany



Shanghai, China



Tokyo, Japan



Background

PCR & NGS-Based Assessment of Clonality in Hematologic Malignancies

Over its 25 year history, Invivoscribe has developed, manufactured, and commercialized the gold-standard molecular hematopathology assays and reagents for gel and capillary electrophoresis detection, and most recently, next-generation sequencing instruments. These standardized, cGMP manufactured assays and reagents were developed and validated using standardized workflow and optimized primer sets, reagents and controls.

A number of our products were developed in collaboration with studies conducted by the EuroClonality BIOMED-2 concerted action group; these capillary based products have provided reliable methods for clonality detection that have withstood the test of time.

We have never accepted the status quo, so our comprehensive menu of clonality assays continues to evolve. All of our NGS-based clonality assays were developed in-house together with accompanying bioinformatics software by our Invivoscribe R&D team. Developed under full ISO 13485 design control, these assays and bioinformatics software were designed to run on several next-generation sequencing platforms. These NGS-based assays are several generations ahead of capillary-based products.

Our comprehensive bioinformatics software not only provides critical information on the presence of clonality, but also identifies the sequence information required to track clones in subsequent samples.

The unique process of gene rearrangement that occurs within the immunoglobulin (*Ig*) and T-cell receptor (*TCR*) gene loci during immune cell development and maturation generates a vast pool of genetically distinct cells. The resulting diverse population of lymphocytes displays an astonishing number of diverse antigen receptors, each coded in the DNA by a unique sequence, and each displayed on the cell surface, or as antibodies in the blood unique to a given cell.^{1,2} This diversity allows the adaptive immune system to carry out its role in protecting the human body by recognizing the infinite number of pathogens it might encounter during a lifetime.

In sum, lymphoid malignancies are characterized by size- and sequence-specific rearrangements within these loci, which result from the transformation and subsequent proliferation from a single cell. The associated cellular population typically shares one or more cell-specific or “clonal” antigen-receptor gene rearrangements. The detection of these clonal cells forms the basis for clonality assessment in leukemia, lymphoma, and hematologic disease. These methods can also be used to assess somatic hypermutation (SHM) and to study minimal residual disease (MRD).

Malignant cells that remain in the bone marrow following treatment are a major cause of disease relapse. MRD testing by NGS offers enhanced sensitivity and specificity (compared to MRD testing by flow cytometry), and allows residual cells to be identified at very low levels and monitored throughout the different stages of disease.

Invivoscribe can provide you with the necessary tools to accommodate your needs. From gel detection to NGS, we can help you accurately identify and track hematologic biomarkers.

For additional information on the detection methods available and the biomarkers offered, please refer to the respective product sections of this catalog.

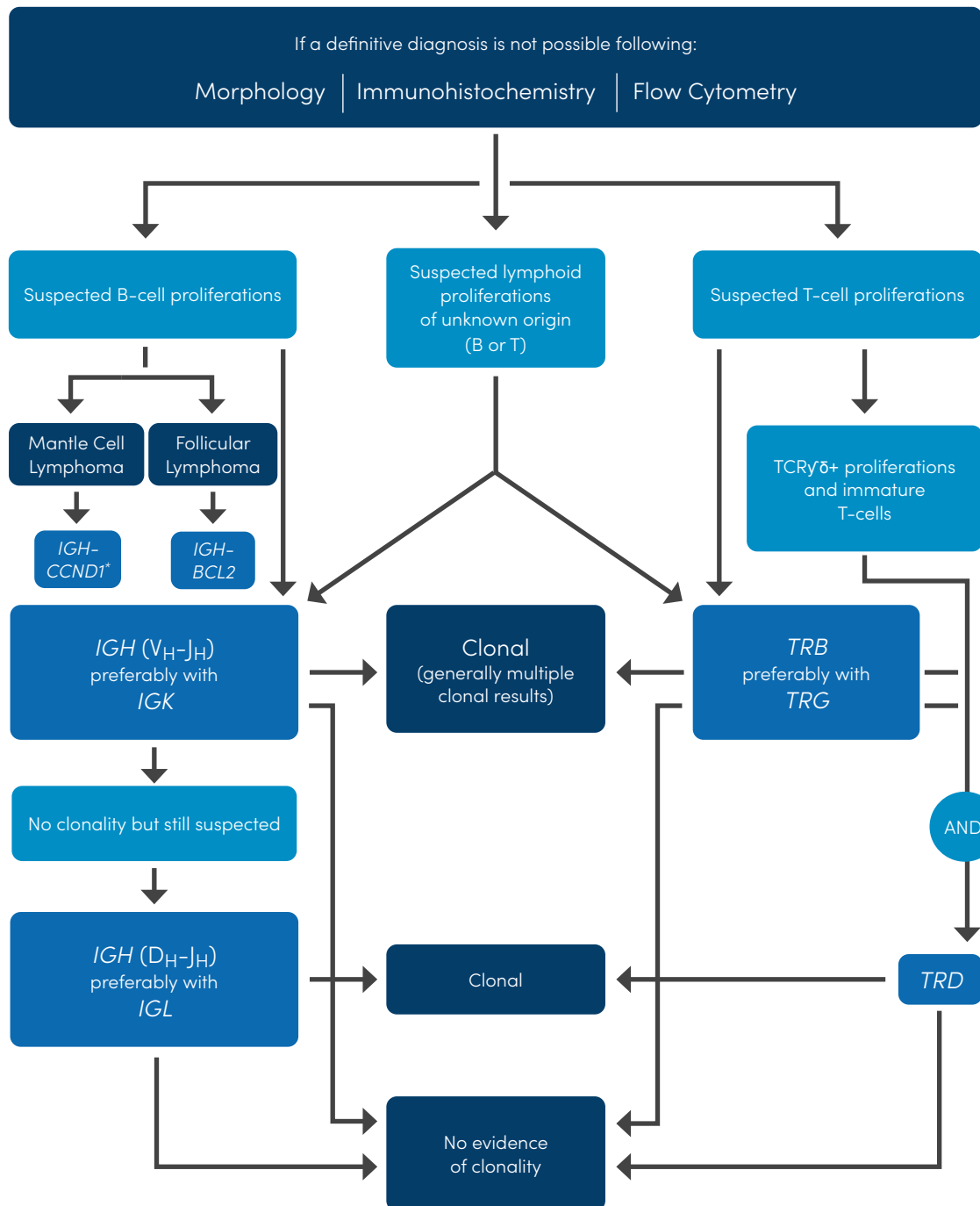
Immunoglobulin and T-Cell Receptor Gene Rearrangements and the Principle and Method of Clonality Testing

The adaptive vertebrate immune system produces a repertoire of immunoglobulin and T-cell receptor molecules using a relatively limited number of heritable germline gene segments. Somatic gene rearrangement is the fundamental mechanism used to generate different immunoglobulin and T-cell receptor molecules, each with unique binding specificity. Lymphocytes undergo gene rearrangements to assemble CDR3 coding regions that are unique in both size and DNA sequence. Since leukemias and lymphomas arise from the malignant transformation of a single cell, they share clonal rearrangement(s) of the antigen receptor genes. This is the basis for clonality testing.³

References | 1. Tonegawa, S. Somatic Generation of Antibody Diversity. *Nature* 302:575-581 (1983). | 2. Expression of T-cell receptor genes during early T-cell development *Immunol Cell Biol.* 2008 Feb;86(2):166-74. Epub 2007 Oct 23. | 3. Miller, J. E. (2013). Principle of Immunoglobulin and T cell Receptor Gene Rearrangement. In Cheng, L., Zhang, D., Eble, J. N. (Eds), *Molecular Genetic Pathology* (2nd Ed., Sections 30.2.7.13 and 30.2.7.18). pp825 – 856. New York, USA: Springer Science & Business Media.

Test Algorithm for Suspect Lymphoproliferations

Developed in concert with the EuroClonality/BIOMED-2 group for PCR-based clonality assessment of suspected B- and T-cell lymphoproliferative disorders.



Hematological Test Menu

Diseases		Gene Rearrangement							Translocation				Mutations		
		IGH V _H - J _H	IGH D _H - J _H	IGK	IGL	IGHV SHM	TRB	TRD	TRG	IGH- BCL1 (CCND1)	IGH- BCL2	BCR- ABL1*	PML RARα*	FLT3	NPM1
Lymphoid/ Lymphoma	Marginal Zone Lymphoma (MZL), extranodal ^{12,13,27}	88%	58%	84%	29%		23%	10%	16%						
	Marginal Zone Lymphoma (MZL), nodal ¹³	100%	30%	80%	30%		10%	20%	10%						
	Mantle Cell Lymphoma (MCL) ^{2,6,7,12,13,27,37}	100%	11%	100%	44%	*	9%	4%	11%	75%					
	Follicular Lymphoma (FL) ^{3,7,12,13,27,28}	84%	19%	84%	21%		6%	5%	2%		90%				
	Diffuse Large B-cell Lymphoma (DLBCL) ^{3,12,13,27}	80%	30%	80%	28%		21%	14%	15%		30%				
	Multiple Myeloma (MM) and other Plasma Cell Neoplasms (PCN) ^{2,9,10,20,25}	84%	60%	57%	97%					20%					
	Chronic Lymphocytic Leukemia (CLL) ^{11,12,15,23,27,35}	100%	43%	100%	30%	*	25%	12%	18%						
	B-cell Acute Lymphoblastic Leukemia (B-ALL) ^{4,12,14,19,21,22,27,29,30,31,32,33,34}	96%	57%	95%	20%		81%	86%	75%			30%			
	Suspect B-cell Proliferations ^{12,26,27,33}	93%	93%	90%	40%		20%		20%						
	Peripheral T-cell Lymphoma (PTCL) ^{12,13,14,24}	35%	4%		2%		98%		94%						
	T-cell Acute Lymphoblastic Leukemia (T-ALL) ^{12,14,21,22,29,31}	24%	25%	4%			92%	68%	95%						
	Angioimmunoblastic T-cell Lymphoma (ALT) ^{12,13,14}	19%	11%	30%	5%		99%	35%	92%						
	Adult T-cell Leukemia/Lymphoma ³⁹						97%		96%						
	Anaplastic Large-Cell Lymphoma (ALCL) ^{12,13,14}						74%	12%	74%						
	T-cell Prolymphocytic Leukemia (T-PLL) ^{12,13,14}	3%	3%	3%	3%		100%	6%	94%						
	T-cell Large Granular Lymphocytic Leukemia (T-LGL Leukemia) ^{12,13,14}			4%	4%		97%	29%	96%						
Suspect T-Cell Proliferations ^{12,26,40}	10%		10%			90%	11%	90%							
Myeloid	Acute Myeloid Leukemia (AML) ^{5,16}													33%	64%
	Acute Promyelocytic Leukemia (APL) ^{1,5,16,17}												90%		
	Chronic Myeloid Leukemia (CML) ^{7,18,19,21,38}											87%			
	Myeloproliferative Neoplasms (MPNs) ³⁸											10%			

Note: The percentage of samples within a given disease category were detected using each gene target. Percentages indicate the highest referenced value.

Hematological Test Menu

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LymphoTrack[®] Dx

NEXT-GENERATION SEQUENCING (NGS) CE-IVD ASSAYS

Key Benefits

- » One-step PCR for amplicon and library generation
- » Identify, track, and assess mutation status of B- and T-cell gene rearrangements
- » Sequence amplicons from any LymphoTrack Dx kit together
- » Included bioinformatics software for easy analysis and interpretation

NGS Assays

- 26** LymphoTrack Dx *IGHV* Leader Somatic Hypermutation Assay
- 28** LymphoTrack Dx *IGH* FR1/2/3 Assays
- 32** LymphoTrack Dx *IGK* Assays
- 34** LymphoTrack Dx *TRG* Assays
- 36** LymphoTrack Dx *TRB* Assay
- 38** LymphoTrack Dx Bioinformatics Software

Warranty and Liability

Inivoscribe, Inc. (Inivoscribe[®]) is committed to providing the highest quality products. Inivoscribe[®] warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Inivoscribe. Inivoscribe liability shall not exceed the purchase price of the product. Inivoscribe shall have no liability for direct, indirect, consequential, or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

Notice

This product is covered by one or more patents owned by or licensed to Inivoscribe, Inc., including US Patent No. 7,785,783, US Patent No. 8,859,748, US Patent No. 10,280,462 European Patent No. 1549764 (validated in 16 countries, and augmented by related European Patent Nos 2418287 and 2460889), European Patent No. 1633884, Japanese Patent No. 4708029, Japanese Patent No. 6189600, Brazilian Patent No. PI0410283, Canadian Patent No. 2525122, Indian Patent No. 243620, Mexican Patent No. 286493, Chinese Patent No. 1806051, and Korean Patent No. 10-1215194.

These products use nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). Any necessary license to practice amplification methods or to use reagents, amplification enzymes or equipment covered by third party patents is the responsibility of the user and no such license is granted by Inivoscribe, Inc., expressly or by implication.

LymphoTrack[®] Dx

LymphoTrack Dx Assay kits are designed for the identification of gene rearrangements in hematologic samples utilizing next-generation sequencing (NGS) technologies.

These assays take advantage of the wealth and depth of data generated by the Illumina[®] MiSeq[™], Thermo Fisher Scientific[®] Ion PGM[™] and Ion S5[™] platforms.

The Invivoscribe NGS assays offer significant improvements over conventional fragment analysis of B- and T-cell gene rearrangements by providing detailed information regarding the DNA sequences, sequence frequency, and mutational status (*IGHV* Leader and *IGH* FR1 only) of each clonotype.

LymphoTrack Dx Assay kits are a complete solution. Kits contain ready-to-use indexed amplification master mixes, necessary controls, and complimentary bioinformatics software. As primers are designed with barcoded indices and adapters, sequencing libraries can be generated with a single PCR, streamlining the overall workflow, eliminating the need for a post-PCR ligation step, and reducing the potential for sample cross contamination.

The per sample testing costs can be reduced by pooling samples from different LymphoTrack Dx Assays into a single sequencing run. The included bioinformatics software will sort the complex NGS data for easy analysis and visualization of individual samples.

Detailed instructions for use are provided with all kits and the Invivoscribe technical support team is always available to answer your questions.

For more information, please visit www.invivoscribe.com

Disclaimer | The LymphoTrack Dx Assays are *in vitro* diagnostic products and are not available for sale or use within North America. For more information regarding the research use only reagents, please see the Next-Generation Sequencing – LymphoTrack section.

LymphoTrack[®] Dx

IGHV Somatic Hypermutation Assay

Assay Description

The LymphoTrack Dx IGHV Leader Somatic Hypermutation Assay for the Illumina[®] MiSeq[™] is an *in vitro* diagnostic product intended for next-generation sequencing (NGS) based determination of the frequency distribution of IGH gene rearrangements, as well as the degree of somatic hypermutation (SHM) of rearranged genes in patients suspected of having lymphoproliferative disease. This assay aids in the identification of lymphoproliferative disorders as well as providing an aid in determining disease prognosis. If you would like to test for IGHV somatic hypermutation using the Thermo Fisher[®] Ion PGM[™] or Ion S5[™] platform, please refer to the LymphoTrack Dx IGH FR1 Assay (9-121-0007).

Summary and Explanation of the Test

The NGS LymphoTrack Dx IGHV Leader Somatic Hypermutation Assay for the Illumina[®] MiSeq[™] represents a significant improvement over clonality assays using fragment analysis as it efficiently detects the majority of IGH gene rearrangements using a single multiplex master mix, identifies the DNA sequence specific for each clonal gene rearrangement, and assesses the somatic hypermutation rate of clonal samples in the same workflow.

The single multiplex master mixes target the Leader (V_HL) and the joining (J_H) gene regions of the IGH locus and are designed with Illumina[®] adapters and indices (8 included in Kit A and 24 included in the Panel). This allows for a one-step PCR reaction and pooling of amplicons from several different samples and targets into a single Illumina[®] MiSeq[™] run. No post-PCR ligation step is required.

The included LymphoTrack Dx Bioinformatics Software enables simplified analysis and visualization of individual sample data.

Positive (clonal positive, SHM negative), negative (polyclonal) and SHM (clonal positive, SHM positive) DNA controls are included in the kits.

Background

The human immunoglobulin heavy chain (IGH) gene locus on chromosome 14 (14q32.3) includes 46–52 functional and 30 nonfunctional variable (V_H) gene segments, 27 functional diversity (D_H) gene segments, and 6 functional joining (J_H) gene segments spread over 1,250 kilobases.

During B-cell development, genes encoding the IGH protein are assembled from multiple polymorphic gene segments that undergo rearrangements and selection, generating cell specific V_H-D_H-J_H rearrangements that are unique in both length and sequence.¹

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect IGH clonal rearrangements can be useful in the study of B- cell malignancies. An additional level of diversity is further generated in the antigen receptors by introducing point mutations in the variable regions, also named SHM. In instances where there is a high degree of SHM, there is the risk that primers located within the variable region will not be able to bind and clonal products will not amplify. In these cases, the leader primers located upstream of the variable region can be beneficial for the detection of clonal products, due to the conserved nature of the V_HL region. In addition, the SHM rate of the entire variable gene can be determined using the V_HL primers.

Determining the immunoglobulin variable heavy chain gene (IGHV) hypermutation rate is considered a gold standard for determining the prognosis of patients with chronic lymphocytic leukemia (CLL)² and small lymphocytic lymphoma (SLL). In addition, NGS methods can improve disease stratification.

Specimen Requirement

50 ng of high-quality genomic DNA.

References

1. Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 302.2.7.13 and 30.2.7.18.
2. Ghia et al., *Leukemia* 21: 2-3 (2007).



Simplified representation of the immunoglobulin heavy chain (IGH) gene locus on chromosome 14. Depicted are the variable (V_H) and downstream consensus joining (J_H) region genes involved in rearrangements. Upstream of the variable gene segments, the leader sequence (V_HL) is also depicted. Diversity region genes are not depicted.

Reagents - MiSeq™ Detection

Kit A Components		Panel Components (includes all master mixes from Kit A plus the items below)			
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
IGH Leader MiSeq 01	A001	IGH Leader MiSeq 09	A009	IGH Leader MiSeq 18	A018
IGH Leader MiSeq 02	A002	IGH Leader MiSeq 10	A010	IGH Leader MiSeq 19	A019
IGH Leader MiSeq 03	A003	IGH Leader MiSeq 11	A011	IGH Leader MiSeq 20	A020
IGH Leader MiSeq 04	A004	IGH Leader MiSeq 12	A012	IGH Leader MiSeq 21	A021
IGH Leader MiSeq 05	A005	IGH Leader MiSeq 13	A013	IGH Leader MiSeq 22	A022
IGH Leader MiSeq 06	A006	IGH Leader MiSeq 14	A014	IGH Leader MiSeq 23	A023
IGH Leader MiSeq 07	A007	IGH Leader MiSeq 15	A015	IGH Leader MiSeq 25	A025
IGH Leader MiSeq 08	A008	IGH Leader MiSeq 16	A016	IGH Leader MiSeq 27	A027
Controls		Controls			
IGH SHM POS (+) Qty. 1		IGH SHM POS (+) Qty. 3			
IGH POS (+) Qty. 1		IGH POS (+) Qty. 3			
NGS NEG (-) Qty. 1		NGS NEG (-) Qty. 3			

Rank	Sequence	Length	Merge count	V-gene	J-gene	% Total reads	Cumulative %	Mutation rate partial V-gene (%)	In-frame (Y/N)	No stop codon (Y/N)	V-coverage
1	TTCTCGTGGTG	455	29603	IGHV4-59_08	IGHJ4_02	9.93	9.93	11.26	Y	Y	98.63
2	CTCGCCCTCCT	463	205	IGHV5-51_01	IGHJ4_02	0.07	9.99	0.00	Y	Y	99.66
3	GGTTTCCTTG	484	201	IGHV3-7_01	IGHJ4_02	0.07	10.06	7.77	Y	Y	100.00
4	CTCGCCCTCCT	463	185	IGHV5-51_01	IGHJ5_02	0.06	10.12	6.08	Y	Y	99.32
5	CTCGCCCTCCT	469	170	IGHV5-51_01	IGHJ4_02	0.06	10.18	0.00	Y	Y	99.32
6	CTCGCCCTCCT	466	160	IGHV5-51_01	IGHJ4_02	0.05	10.23	0.00	Y	Y	99.66
7	CTGCTGCTGAC	460	159	IGHV2-5_10	IGHJ5_02	0.05	10.29	8.08	Y	Y	97.64
8	GGTTTCCTTG	493	156	IGHV3-48_02	IGHJ6_02	0.05	10.34	3.72	Y	Y	98.99
9	CTCGCCCTCCT	334	153	IGHV5-51_02	IGHJ2_01	0.05	10.39	3.72	Y	N	27.70
10	CTCGCCCTCCT	334	152	IGHV5-51_02	IGHJ2_01	0.05	10.44	3.38	Y	N	26.01

Example Data. The top 10 sequences from a read summary generated by the LymphoTrack Dx Software - MiSeq™ with the SHM mutation rate and predictions pertaining to whether a sequence is in-frame or contains a premature stop codon are depicted. To learn more about the LymphoTrack Dx software, please refer to the LymphoTrack Dx Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
9-121-0059	LymphoTrack® Dx IGHV Leader Somatic Hypermutation Assay Kit A - MiSeq™	8 indices - 5 sequencing reactions each
9-121-0069	LymphoTrack® Dx IGHV Leader Somatic Hypermutation Assay Panel - MiSeq™	24 indices - 5 sequencing reactions each
9-500-0009	LymphoTrack® Dx Software - MiSeq™	1 CD complimentary with purchase

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use and available in regions that accept CE-IVD products.

LymphoTrack[®] Dx

IGH FR1/2/3 Assays

Assay Description

LymphoTrack Dx IGH FR1 Assays

The LymphoTrack Dx IGH FR1 Assay for the Illumina MiSeq[™] or Thermo Fisher Scientific[®] Ion S5[™] and Ion PGM[™] is an *in vitro* diagnostic product intended for next-generation sequencing (NGS) based determination of the frequency distribution of IGH gene rearrangements as well as the degree of somatic hypermutation of rearranged genes in patients suspected with having lymphoproliferative disease. This assay aids in the identification of lymphoproliferative disorders as well as providing an aid in determining disease prognosis.

LymphoTrack Dx IGH FR1/2/3 Assays

The LymphoTrack Dx IGH FR1 Assay for the Illumina MiSeq[™] or Thermo Fisher Scientific[®] Ion S5[™] and Ion PGM[™] is an *in vitro* diagnostic product intended for next-generation sequencing (NGS) based determination of the frequency distribution of IGH gene rearrangements as well as the degree of somatic hypermutation of rearranged genes in patients suspected with having lymphoproliferative disease. This assay aids in the identification of lymphoproliferative disorders as well as providing an aid in determining disease prognosis.

This LymphoTrack Dx IGH FR2 Assay is an *in vitro* diagnostic product intended for next-generation sequencing (NGS) for the Illumina MiSeq[™] or Thermo Fisher Scientific[®] Ion S5[™] and Ion PGM[™] instruments. The assay will determine the frequency distribution of IGH V_H-J_H gene rearrangements in patients suspected with having lymphoproliferative disease. This assay aids in the identification of lymphoproliferative disorders.

The LymphoTrack Dx IGH FR3 Assay is an *in vitro* diagnostic product intended for next-generation sequencing (NGS) for the Illumina MiSeq[™] or Thermo Fisher Scientific[®] Ion S5[™] and Ion PGM[™] instruments. The assay will determine the frequency distribution of IGH V_H-J_H gene rearrangements in patients suspected with having lymphoproliferative disease. This assay aids in the identification of lymphoproliferative disorders.

Summary and Explanation of the Test

The LymphoTrack Dx IGH Assays represent a significant improvement over conventional clonality assessment methods utilizing fragment analysis by providing four important and complementary uses in a single workflow:

1. Detection of clonal populations.
2. Identification of sequence information and gene segment utilization.
3. The LymphoTrack Dx IGH framework 1 FR1 master mixes provide the degree of SHM in the immunoglobulin variable heavy chain (IGHV) gene locus.
4. The ability to track sequences in subsequent samples with the Invivoscribe LymphoTrack MRD* Software.

These assays utilize a single multiplex master mix to target each conserved IGH Framework Region (FR1, FR2, and FR3) within the V_H and the J_H regions described in lymphoid malignancies. Each single multiplex master mix targets one of the conserved IGH framework regions (FR1, FR2, or FR3) within the V_H and the J_H regions described in lymphoid malignancies. Targeting all three framework regions significantly reduces the risk of not being able to detect the presence of clonality, as somatic hypermutations in the primer binding sites of the involved V_H gene segments can impede DNA amplification.¹ The included primers are designed with Illumina[®] or Thermo Fisher Scientific adapters and indices (8-24 and 12, respectively). This allows up to 24 samples on MiSeq[™] and 12 samples on Ion PGM[™] and Ion S5[™] to be sequenced at the same time with any of the individual FRs.

In addition, amplicons generated with different FR master mixes or Invivoscribe LymphoTrack Dx kits (such as IGH or TRG) can be pooled together in the same sequencing library to reduce testing costs. The associated LymphoTrack Dx Software provides interpretation of the data via a simple and streamlined method of analysis and visualization. By following the guidelines provided in the instructions for use, samples can be interpreted for evidence of clonality and SHM rates.

Positive clonal (SHM negative) and negative polyclonal DNA controls are included in kits. A clonal SHM positive control can be purchased separately (cat#: 4-088-0008).

Background

The human immunoglobulin heavy chain (IGH) gene locus on chromosome 14 (14q32.3) includes 46-52 functional and 30 non-functional variable (V_H), 27 functional diversity (D_H), and 6 functional joining (J_H) gene segments. The V_H gene segments can be further broken down into three conserved frameworks (FR) and three variable complementarity-determining regions (CDRs).

During development of lymphoid cells, antigen receptor genes undergo somatic gene rearrangements.² Specifically during B-cell development, IGH molecules are assembled from multiple polymorphic gene segments that undergo rearrangements generating V_H-D_H-J_H combinations unique in both length and sequence.³ Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements.

In addition, the IGHV hypermutation status obtained with the LymphoTrack Dx IGH FR1 master mixes, provides important prognostic information for patients with chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). The SHM rate has been shown to have clinical relevance for CLL, as there is a clear distinction in the median survival of patients with and without SHM.⁴

*MRD Software can be used to track sequences generated by either LymphoTrack Assays - MiSeq[™] or Ion S5/PGM[™]. MRD applications are for Research Use Only.

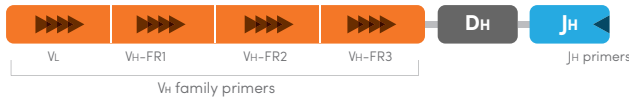
Specimen Requirement

50 ng of high-quality genomic DNA.

References

1. Evans, P. A. et al., (2007). *Leukemia* 21, 207-14.
2. Tonegawa, S. (1983). *Nature* 302, 575-581.
3. Miller JE. (2013) *Molecular Genetic Pathology* (2nd Edition, sections 30.2.7.13 and 30.2.7.18).
4. Chia et al., *Blood* 105:1678-1685 (2005).

Simplified Representation of the IGH Gene



Simplified depiction of variable (V_H) and downstream consensus joining (J_H) region genes involved in gene rearrangements.

Reagents - MiSeq™ Detection

The LymphoTrack Dx IGH FR1/2/3 Assays contain components from respective individual FR kit A's or panels.

LymphoTrack Dx IGH FR1 Components		LymphoTrack Dx IGH FR2 Components		LymphoTrack Dx IGH FR3 Components	
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
IGH FR1 MiSeq 01	A001	IGH FR2 MiSeq 01	A001	IGH FR3 MiSeq 01	A001
IGH FR1 MiSeq 02	A002	IGH FR2 MiSeq 02	A002	IGH FR3 MiSeq 02	A002
IGH FR1 MiSeq 03	A003	IGH FR2 MiSeq 03	A003	IGH FR3 MiSeq 03	A003
IGH FR1 MiSeq 04	A004	IGH FR2 MiSeq 04	A004	IGH FR3 MiSeq 04	A004
IGH FR1 MiSeq 05	A005	IGH FR2 MiSeq 05	A005	IGH FR3 MiSeq 05	A005
IGH FR1 MiSeq 06	A006	IGH FR2 MiSeq 06	A006	IGH FR3 MiSeq 06	A006
IGH FR1 MiSeq 07	A007	IGH FR2 MiSeq 07	A007	IGH FR3 MiSeq 07	A007
IGH FR1 MiSeq 08	A008	IGH FR2 MiSeq 08	A008	IGH FR3 MiSeq 08	A008
IGH FR1 MiSeq 09	A009	IGH FR2 MiSeq 09	A009	IGH FR3 MiSeq 09	A009
IGH FR1 MiSeq 10	A010	IGH FR2 MiSeq 10	A010	IGH FR3 MiSeq 10	A010
IGH FR1 MiSeq 11	A011	IGH FR2 MiSeq 11	A011	IGH FR3 MiSeq 11	A011
IGH FR1 MiSeq 12	A012	IGH FR2 MiSeq 12	A012	IGH FR3 MiSeq 12	A012
IGH FR1 MiSeq 13	A013	IGH FR2 MiSeq 13	A013	IGH FR3 MiSeq 13	A013
IGH FR1 MiSeq 14	A014	IGH FR2 MiSeq 14	A014	IGH FR3 MiSeq 14	A014
IGH FR1 MiSeq 15	A015	IGH FR2 MiSeq 15	A015	IGH FR3 MiSeq 15	A015
IGH FR1 MiSeq 16	A016	IGH FR2 MiSeq 16	A016	IGH FR3 MiSeq 16	A016
IGH FR1 MiSeq 18	A018	IGH FR2 MiSeq 18	A018	IGH FR3 MiSeq 18	A018
IGH FR1 MiSeq 19	A019	IGH FR2 MiSeq 19	A019	IGH FR3 MiSeq 19	A019
IGH FR1 MiSeq 20	A020	IGH FR2 MiSeq 20	A020	IGH FR3 MiSeq 20	A020
IGH FR1 MiSeq 21	A021	IGH FR2 MiSeq 21	A021	IGH FR3 MiSeq 21	A021
IGH FR1 MiSeq 22	A022	IGH FR2 MiSeq 22	A022	IGH FR3 MiSeq 22	A022
IGH FR1 MiSeq 23	A023	IGH FR2 MiSeq 23	A023	IGH FR3 MiSeq 23	A023
IGH FR1 MiSeq 25	A025	IGH FR2 MiSeq 25	A025	IGH FR3 MiSeq 25	A025
IGH FR1 MiSeq 27	A027	IGH FR2 MiSeq 27	A027	IGH FR3 MiSeq 27	A027

Kit A's contain indices IGH FRX A001 to A008. Panels contain all master mixes listed above.

Controls in Individual FR (1, 2, or 3) Kit A's	Controls in Individual FR (1, 2, or 3) Panels	Controls in Combo FR 1/2/3 Kit A	Controls in Combo FR 1/2/3 Panel
IGH POS (+) Qty. 1	IGH POS (+) Qty 3	IGH POS (+) Qty. 2	IGH POS (+) Qty. 6
NGS NEG (-) Qty. 1	NGS NEG (-) Qty 3	NGS NEG (-) Qty. 2	NGS NEG (-) Qty. 6

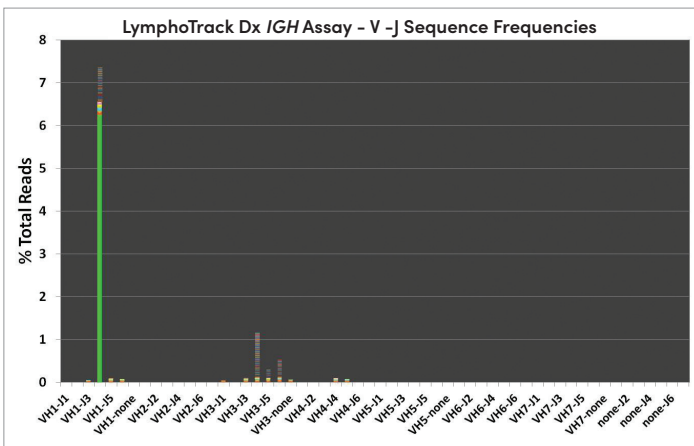
*MRD Software can be used to track sequences generated by either LymphoTrack Assays - MiSeq™ or Ion S5/PGM™. MRD applications are for Research Use Only.

IGH FR1/2/3 Assays Continued

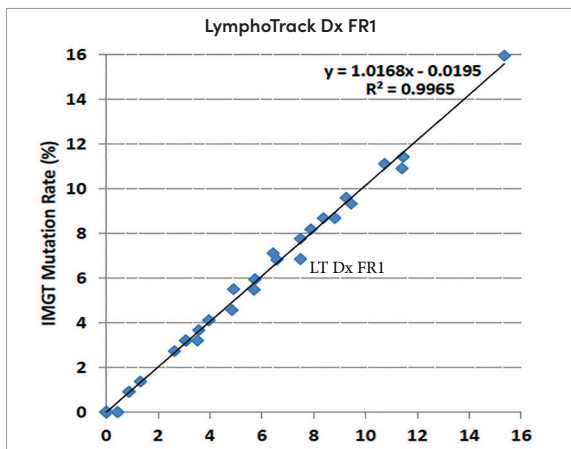
Reagents - Ion S5[™] / PGM[™] Detection

The LymphoTrack Dx IGH FR1/2/3 Assays contain components from respective individual FR Assays.

LymphoTrack Dx IGH FR1 Components		LymphoTrack Dx IGH FR2 Components		LymphoTrack Dx IGH FR3 Components	
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
IGH FR1 Ion S5/PGM 01	IonXpress_001	IGH FR2 Ion S5/PGM 01	IonXpress_001	IGH FR3 Ion S5/PGM 01	IonXpress_001
IGH FR1 Ion S5/PGM 02	IonXpress_002	IGH FR2 Ion S5/PGM 02	IonXpress_002	IGH FR3 Ion S5/PGM 02	IonXpress_002
IGH FR1 Ion S5/PGM 03	IonXpress_003	IGH FR2 Ion S5/PGM 03	IonXpress_003	IGH FR3 Ion S5/PGM 03	IonXpress_003
IGH FR1 Ion S5/PGM 04	IonXpress_004	IGH FR2 Ion S5/PGM 04	IonXpress_004	IGH FR3 Ion S5/PGM 04	IonXpress_004
IGH FR1 Ion S5/PGM 07	IonXpress_007	IGH FR2 Ion S5/PGM 07	IonXpress_007	IGH FR3 Ion S5/PGM 07	IonXpress_007
IGH FR1 Ion S5/PGM 08	IonXpress_008	IGH FR2 Ion S5/PGM 08	IonXpress_008	IGH FR3 Ion S5/PGM 08	IonXpress_008
IGH FR1 Ion S5/PGM 09	IonXpress_009	IGH FR2 Ion S5/PGM 09	IonXpress_009	IGH FR3 Ion S5/PGM 09	IonXpress_009
IGH FR1 Ion S5/PGM 10	IonXpress_010	IGH FR2 Ion S5/PGM 10	IonXpress_010	IGH FR3 Ion S5/PGM 10	IonXpress_010
IGH FR1 Ion S5/PGM 11	IonXpress_011	IGH FR2 Ion S5/PGM 11	IonXpress_011	IGH FR3 Ion S5/PGM 11	IonXpress_011
IGH FR1 Ion S5/PGM 12	IonXpress_012	IGH FR2 Ion S5/PGM 12	IonXpress_012	IGH FR3 Ion S5/PGM 12	IonXpress_012
IGH FR1 Ion S5/PGM 13	IonXpress_013	IGH FR2 Ion S5/PGM 13	IonXpress_013	IGH FR3 Ion S5/PGM 13	IonXpress_013
IGH FR1 Ion S5/PGM 14	IonXpress_014	IGH FR2 Ion S5/PGM 14	IonXpress_014	IGH FR3 Ion S5/PGM 14	IonXpress_014
Controls in Individual FR (1,2, or 3) Kits			Controls in FR 1/2/3 Kit		
IGH POS (+) Qty. 2			IGH POS (+) Qty. 4		
NGS NEG (-) Qty. 2			NGS NEG (-) Qty. 4		



V-J Sequence Frequencies. The LymphoTrack Dx Software provides a stacked bar graph depicting the relative frequencies of the 200 most prevalent VH-JH rearrangements identified in a sample. To learn more about the LymphoTrack Dx software, please refer to the LymphoTrack Dx Bioinformatics Software section.



Comparison of SHM Analysis Methods. The SHM rate of 51 CLL samples was determined by the LymphoTrack Dx *IGH* FR1 Assay – MiSeq™ and analyzed with both the LymphoTrack Dx Software – MiSeq™ and IMGT analysis.

Reagents – Miseq™ Detection

Ordering Information

Catalog #	Products	Quantity Components
9-121-0129	LymphoTrack® Dx <i>IGH</i> FR1/2/3 Assay Kit A – MiSeq™	8 + 8 + 8 indices – 5 sequencing reactions each
9-121-0139	LymphoTrack® Dx <i>IGH</i> FR1/2/3 Assay Panel – MiSeq™	24 + 24 + 24 indices – 5 sequencing reactions each
9-121-0009	LymphoTrack® Dx <i>IGH</i> FR1 Assay Kit A – MiSeq™	8 indices – 5 sequencing reactions each
9-121-0039	LymphoTrack® Dx <i>IGH</i> FR1 Assay Panel – MiSeq™	24 indices – 5 sequencing reactions each
9-121-0089	LymphoTrack® Dx <i>IGH</i> FR2 Assay Kit A – MiSeq™	8 indices – 5 sequencing reactions each
9-121-0099	LymphoTrack® Dx <i>IGH</i> FR2 Assay Panel – MiSeq™	24 indices – 5 sequencing reactions each
9-121-0109	LymphoTrack® Dx <i>IGH</i> FR3 Assay Kit A – MiSeq™	8 indices – 5 sequencing reactions each
9-121-0119	LymphoTrack® Dx <i>IGH</i> FR3 Assay Panel – MiSeq™	24 indices – 5 sequencing reactions each
9-500-0009	LymphoTrack® Dx Software – MiSeq™	1 CD complimentary with purchase

Reagents – Ion S5™ / PGM™ Detection

Ordering Information

Catalog #	Products	Quantity Components
9-121-0057	LymphoTrack® Dx <i>IGH</i> FR1/2/3 Assay – S5/PGM™	12 + 12 + 12 indices – 5 sequencing reactions each
9-121-0007	LymphoTrack® Dx <i>IGH</i> FR1 Assay – S5/PGM™	12 indices – 5 sequencing reactions each
9-121-0037	LymphoTrack® Dx <i>IGH</i> FR2 Assay – S5/PGM™	12 indices – 5 sequencing reactions each
9-121-0047	LymphoTrack® Dx <i>IGH</i> FR3 Assay – S5/PGM™	12 indices – 5 sequencing reactions each
9-500-0007	LymphoTrack® Dx Software – S5/PGM™	1 CD complimentary with purchase

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use and available in regions that accept CE-IVD products.

LymphoTrack[®] Dx

IGK Assay

Assay Description

The LymphoTrack Dx *IGK* Assays for the Illumina[®] MiSeq[™], or Thermo Fisher Scientific[®] Ion PGM[™] and Ion S5[™] instruments are *in vitro* diagnostic products intended for next-generation sequencing (NGS) based determination of the frequency distribution of *IGK* gene rearrangements in patients suspected of having lymphoproliferative disease. These assays aid in the identification of lymphoproliferative disorders.

Summary and Explanation of the Test

In contrast to the IdentiClone[®] fragment analysis assays for clonality that utilize two master mixes, these NGS assays contain a single multiplex master mix to target conserved regions of the *IGK* gene locus described in lymphoid malignancies, thereby, reducing sample DNA requirements and simplifying the testing workflow. The LymphoTrack Dx *IGK* master mix primers are also designed with Illumina[®] or Thermo Fisher Scientific adapters and up to 24 different indices. This allows amplicons generated from different indexed *IGK* master mixes to be pooled into a single library for loading onto one MiSeq[™] flow cell, Ion PGM[™] or Ion S5[™] chips.

The associated LymphoTrack Dx Software is capable of sorting complex NGS data by gene target, providing users the ability to reduce per sample testing costs by sequencing amplicons from any LymphoTrack Dx Assay (e.g. *IGH*, *IGK*, *TRB*, *TRG*) at the same time. In addition, the LymphoTrack Dx Software provides an easy and streamlined method for visualization of data and guidelines provided in the instructions for use allow samples to be interpreted for evidence or no evidence of clonality.

Positive clonal and negative polyclonal DNA controls are included in kits.

Background

The LymphoTrack Dx *IGK* Assays represent a significant improvement over existing fragment analysis clonality assays by providing two important and complementary uses:

1. Detection of clonal populations.
2. Identification of sequence information and gene segment utilization.
3. Ability to track sequences in subsequent samples with the use of the Invivoscribe LymphoTrack MRD Software.

The human immunoglobulin kappa (*IGK*) gene locus on chromosome 2 (2p11.2) includes 76 V (variable) region genes spanning 7 subgroups and 5 J (joining) region gene segments upstream of the (C_κ) region.

During lymphoid cell development, antigen receptor genes undergo somatic gene rearrangements.¹ Specifically, during B-cell development, genes encoding *IGK* molecules are assembled from multiple polymorphic gene segments that generate V-J combinations unique in both length and sequence.²

In addition, the kappa deleting element (K_{de}), approximately 24 kb downstream of the J_κ-C_κ region can also rearrange with V_κ gene segments and the isolated recombination signal sequence in the J_κ-C_κ intron (J_κ-C_κ INTR).

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or “clonal” antigen receptor gene rearrangements. Therefore, tests that detect *IGK* clonal rearrangements can be useful in the study of B- cell malignancies and complement *IGH* testing, as the *IGK* receptor is less susceptible to somatic mutations.

Specimen Requirement

50 ng of high-quality genomic DNA.

References

1. Tonegawa, S. (1983). *Nature* 302, 575-581.
2. Miller JE. (2013) *Molecular Genetic Pathology* (2nd Edition, sections 30.2.7.13 and 30.2.7.18).

Simplified Representation of the *IGK* Gene



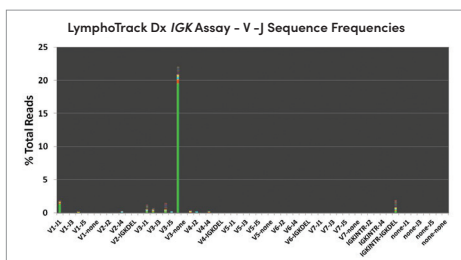
Depicted are the variable region (V_κ) or variable intragenic J_κ-C_κ intron (J_κ-C_κ INTR) genes involved in *IGK* gene rearrangements in addition to the downstream consensus joining region genes (J_κ) or kappa deleting element (K_{de}).

Reagents - MiSeq™ Detection

Kit A Components		Panel Components (includes all master mixes from Kit A plus the items below)			
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
IGK MiSeq 01	A001	IGK MiSeq 09	A009	IGK MiSeq 18	A018
IGK MiSeq 02	A002	IGK MiSeq 10	A010	IGK MiSeq 19	A019
IGK MiSeq 03	A003	IGK MiSeq 11	A011	IGK MiSeq 20	A020
IGK MiSeq 04	A004	IGK MiSeq 12	A012	IGK MiSeq 21	A021
IGK MiSeq 05	A005	IGK MiSeq 13	A013	IGK MiSeq 22	A022
IGK MiSeq 06	A006	IGK MiSeq 14	A014	IGK MiSeq 23	A023
IGK MiSeq 07	A007	IGK MiSeq 15	A015	IGK MiSeq 25	A025
IGK MiSeq 08	A008	IGK MiSeq 16	A016	IGK MiSeq 27	A027
Controls		Controls			
IGK POS (+) Qty. 1	NGS NEG (-) Qty. 1	IGK POS (+) Qty. 3		NGS NEG (-) Qty. 3	

Reagents - Ion S5™/PGM™ Detection

Assay Components			
Master Mix Name	Index #	Master Mix Name	Index #
IGK S5/PGM 01	IonXpress_001	IGK S5/PGM 11	IonXpress_011
IGK S5/PGM 02	IonXpress_002	IGK S5/PGM 12	IonXpress_012
IGK S5/PGM 04	IonXpress_004	IGK S5/PGM 13	IonXpress_013
IGK S5/PGM 08	IonXpress_008	IGK S5/PGM 14	IonXpress_014
IGK S5/PGM 09	IonXpress_009	IGK S5/PGM 16	IonXpress_016
IGK S5/PGM 010	IonXpress_010	IGK S5/PGM 17	IonXpress_017
Controls			
IGK POS (+) Qty. 2		NGS NEG (-) Qty. 2	



V-J Sequence Frequencies. The LymphoTrack Dx Software provides a stacked bar graph depicting the relative frequencies for the most prevalent rearrangements identified in a sample. To learn more about the LymphoTrack Dx software, please refer to the LymphoTrack Dx Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
9-122-0009	LymphoTrack® Dx IGK Assay Kit A - MiSeq™	8 indices - 5 sequencing reactions each
9-122-0019	LymphoTrack® Dx IGK Assay Panel - MiSeq™	24 indices - 5 sequencing reactions each
9-500-0009	LymphoTrack® Dx Software - MiSeq™	1 CD complimentary with purchase
9-122-0007	LymphoTrack® Dx IGK Assay - S5/PGM™	12 indices - 5 sequencing reactions each
9-500-0007	LymphoTrack® Dx Software - S5/PGM™	1 CD complimentary with purchase

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use and available in regions that accept CE-IVD products.

LymphoTrack[®] Dx

TRG Assay

Assay Description

The LymphoTrack Dx TRG Assays for the Illumina[®] MiSeq[™], Thermo Fisher Scientific[®] Ion PGM[™] or Ion S5[™] instruments are *in vitro* diagnostic products intended for next-generation sequencing (NGS) based determination of the frequency distribution of TRG gene rearrangements in patients suspected with having lymphoproliferative disease. These assays aid in the identification of lymphoproliferative disorders.

Summary and Explanation of the Test

This assay utilizes a single multiplex master mix to target conserved V and J regions of the human TRB gene locus described in lymphoid malignancies, thereby reducing sample DNA requirements and simplifying the testing workflow. Primers are designed with Illumina[®] or Thermo Fisher Scientific adapters and up to 24 different indices; thereby allowing amplicons generated from different TRG master mixes to be pooled together for sequencing on a single MiSeq[™] flow cell, or Ion PGM[™] or Ion S5[™] chip.

The associated LymphoTrack Dx Software sorts complex NGS data by gene target, providing users the ability to reduce per sample testing costs by sequencing amplicons generated with any LymphoTrack Dx Assay (e.g. IGH, IGK, TRB, TRG) at the same time. In addition, the LymphoTrack Dx Software provides an easy and streamlined method for data visualization and guidelines provided in the instructions for use allow samples to be interpreted for evidence or no evidence of clonality.

Positive clonal and negative polyclonal DNA controls are included in kits.

Background

The LymphoTrack Dx TRG Assays represent a significant improvement over existing fragment analysis clonality assays by providing two important and complementary uses:

1. Detection of initial clonal populations.
2. Identification of sequence information required to track clonal rearrangements in subsequent samples.

The TRG gene locus on chromosome 7 (7q14) includes 14 V (variable region) genes (Group I, II, III, and IV), 5 J (joining region) gene segments, and 2 C (constant region) genes spread over 200 kilobases.

During development of lymphoid cells, antigen receptor genes undergo somatic gene rearrangements.¹ Specifically during T-cell development, genes encoding TRG molecules are assembled from multiple polymorphic gene segments that undergo rearrangement generating V-J combinations unique in both length and sequence.² Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangement. Therefore, tests that detect TRG clonal rearrangements can be useful in the study of B- and T-cell malignancies.

Note: For a more thorough explanation of the locus and the targeted deep sequencing strategy, please refer to Principle of Immunoglobulin and T-Cell Receptor Gene Rearrangement.²

Specimen Requirement

50 ng of high-quality genomic DNA.

References

1. Tonegawa, S. (1983). *Nature* 302, 575-581.
2. Miller JE. (2013) *Molecular Genetic Pathology* (2nd Edition), sections 30.2.7.13 and 30.2.7.18).

Simplified Representation of the TRG Gene



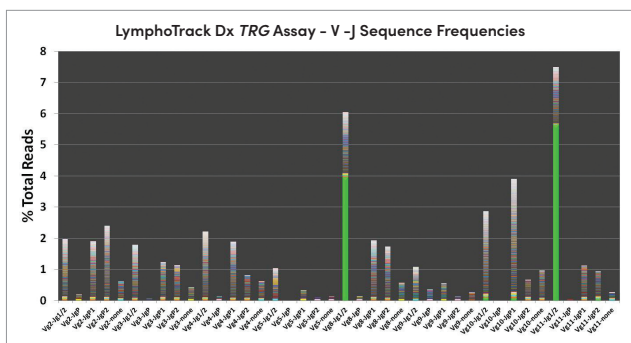
Depicted are the variable region (V) genes and downstream consensus joining region genes (J) that are involved in TRG gene rearrangements.

Reagents - MiSeq™ Detection

Kit A Components		Panel Components (includes all master mixes from Kit A plus the items below)			
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
TRG MiSeq 01	A001	TRG MiSeq 09	A009	TRG MiSeq 18	A018
TRG MiSeq 02	A002	TRG MiSeq 10	A010	TRG MiSeq 19	A019
TRG MiSeq 03	A003	TRG MiSeq 11	A011	TRG MiSeq 20	A020
TRG MiSeq 04	A004	TRG MiSeq 12	A012	TRG MiSeq 21	A021
TRG MiSeq 05	A005	TRG MiSeq 13	A013	TRG MiSeq 22	A022
TRG MiSeq 06	A006	TRG MiSeq 14	A014	TRG MiSeq 23	A023
TRG MiSeq 07	A007	TRG MiSeq 15	A015	TRG MiSeq 25	A025
TRG MiSeq 08	A008	TRG MiSeq 16	A016	TRG MiSeq 27	A027
Controls		Controls			
TRG POS (+) Qty. 1	NGS NEG (-) Qty. 1	TRG POS (+) Qty. 3		NGS NEG (-) Qty. 3	

Reagents - Ion S5™/PGM™ Detection

Assay components					
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
TRG S5/PGM 01	IonXpress_001	TRG S5/PGM 07	IonXpress_007	TRG S5/PGM 11	IonXpress_011
TRG S5/PGM 02	IonXpress_002	TRG S5/PGM 08	IonXpress_008	TRG S5/PGM 12	IonXpress_012
TRG S5/PGM 03	IonXpress_003	TRG S5/PGM 09	IonXpress_009	TRG S5/PGM 13	IonXpress_013
TRG S5/PGM 04	IonXpress_004	TRG S5/PGM 10	IonXpress_010	TRG S5/PGM 14	IonXpress_014
Controls					
TRG POS (+) Qty. 2		NGS NEG (-) Qty. 2			



V-J Sequence Frequencies. The LymphoTrack Dx bioinformatics software provides PDF reports which include Top 10 Merged Read Summary as well as a stacked bar graph depicting the relative frequencies of the V-J rearrangements identified in the sample. To learn more about the LymphoTrack Dx Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
9-227-0019	LymphoTrack® Dx TRG Assay Kit A - MiSeq™	8 indices - 5 sequencing reactions each
9-227-0009	LymphoTrack® Dx TRG Assay Panel - MiSeq™	24 indices - 5 sequencing reactions each
9-500-0009	LymphoTrack® Dx Software - MiSeq™	1 CD complimentary with purchase
9-227-0007	LymphoTrack® Dx TRG Assay - S5/PGM™	12 indices - 5 sequencing reactions each
9-500-0007	LymphoTrack® Dx Software - S5/PGM™	1 CD complimentary with purchase

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use and available in regions that accept CE-IVD products.

LymphoTrack[®] Dx

TRB Assay

Assay Description

The LymphoTrack[®] Dx TRB Assay for the Illumina MiSeq™ is an *in vitro* diagnostic product intended for next-generation sequencing (NGS) based determination of the frequency distribution of TRB gene rearrangements in patients suspected of having lymphoproliferative disease. This assay aids in the identification of lymphoproliferative disorders.

Summary and Explanation of the Test

This assay utilizes a single multiplex master mix to target conserved V and J regions of the human TRB gene locus described in lymphoid malignancies, thereby reducing sample DNA requirements and simplifying the testing workflow. The LymphoTrack Dx TRB master mix primers are designed with Illumina[®] adapters and 8 (Kit A) or 24 (Panel) different indices. This allows amplicons generated from different indexed TRB master mixes to be pooled into a single library for loading onto one MiSeq™ flow cell.

The associated LymphoTrack Dx Software is capable of sorting complex NGS data by gene target, offering a second layer of multiplexing. This provides users the ability to reduce per sample testing costs by sequencing amplicons from any LymphoTrack Dx MiSeq™ Assay (e.g. TRB, TRG, IGH, IGK) at the same time. In addition, the LymphoTrack Dx Software provides an easy and streamlined method for visualization of data. Guidelines to interpret samples for evidence or no evidence of clonality are included in the instructions for use provided with each kit.

Positive clonal and negative polyclonal DNA controls are included in kits.

Background

The LymphoTrack Dx TRB Assays represent a significant improvement over fragment analysis methods for clonality testing by providing two important and complementary uses:

1. Detection of initial clonal populations.
2. Identification of sequence information required to track clonal rearrangements in subsequent samples.

Analysis of the rearranged TRB locus increases the probability of identifying T cell receptor gene rearrangements, as compared to testing for TRG gene rearrangements only. As a result, combining the analysis of TRB and TRG loci increases the sensitivity of clonality detection.

The human T-cell receptor beta (TRB) gene locus on chromosome 7 (7q34) includes 65 V β (variable) gene segments, followed by two separate clusters of genes each containing a D β (diversity) gene, several J β (joining) genes, and a C β (constant) region spread over 685 kilobases. The 2 C β genes, TRBC1 and TRBC2, encode highly homologous products with no functional difference.

During lymphoid cell development, antigen receptor genes undergo somatic gene rearrangements.¹ Specifically, during T-cell development genes encoding TRB molecules are assembled from multiple polymorphic gene segments that generate V β - D β - J β combinations unique in both length and sequence.²

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect TRB clonal rearrangements can be useful in the study of B- and T-cell malignancies.

Note: For a more thorough explanation of the locus and the targeted deep sequencing strategy, please refer to Principle of Immunoglobulin and T-Cell Receptor Gene Rearrangement.²

Specimen Requirement

50 ng of high-quality genomic DNA.

References

1. Tonegawa, S. (1983). *Nature* 302, 575-581.
2. Miller JE. (2013) *Molecular Genetic Pathology* (2nd Edition, sections 30.2.7.13 and 30.2.7.18).
3. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 30.2.7.13.

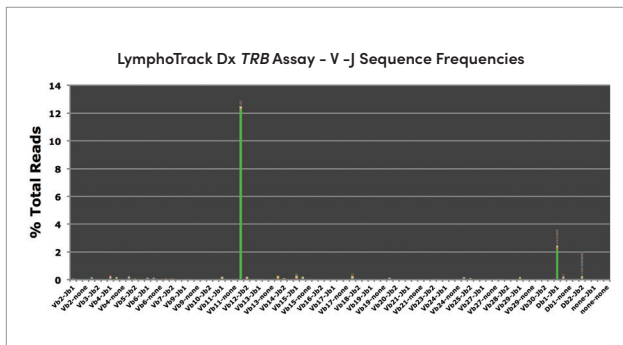
Simplified Representation of the *TRB* Gene



Depicted are the variable (V β), diversity (D β), and joining (J β) gene regions involved in *TRB* gene rearrangements, in addition to the downstream consensus (C β) gene regions.

Reagents - MiSeq™ Detection

Kit A Components		Panel Components (includes all master mixes from Kit A plus the items below)			
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
<i>TRB</i> MiSeq 01	A001	<i>TRB</i> MiSeq 09	A009	<i>TRB</i> MiSeq 18	A018
<i>TRB</i> MiSeq 02	A002	<i>TRB</i> MiSeq 10	A010	<i>TRB</i> MiSeq 19	A019
<i>TRB</i> MiSeq 03	A003	<i>TRB</i> MiSeq 11	A011	<i>TRB</i> MiSeq 20	A020
<i>TRB</i> MiSeq 04	A004	<i>TRB</i> MiSeq 12	A012	<i>TRB</i> MiSeq 21	A021
<i>TRB</i> MiSeq 05	A005	<i>TRB</i> MiSeq 13	A013	<i>TRB</i> MiSeq 22	A022
<i>TRB</i> MiSeq 06	A006	<i>TRB</i> MiSeq 14	A014	<i>TRB</i> MiSeq 23	A023
<i>TRB</i> MiSeq 07	A007	<i>TRB</i> MiSeq 15	A015	<i>TRB</i> MiSeq 25	A025
<i>TRB</i> MiSeq 08	A008	<i>TRB</i> MiSeq 16	A016	<i>TRB</i> MiSeq 27	A027
Controls		Controls			
<i>TRB</i> POS (+) Qty. 1	NGS NEG (-) Qty. 1	<i>TRB</i> POS (+) Qty. 3	NGS NEG (-) Qty. 1	NGS NEG (-) Qty. 3	



V-J Sequence Frequencies. The LymphoTrack Dx bioinformatics software provides a stacked bar graph depicting the relative frequencies for the 200 most prevalent rearrangements sequenced and identified in the sample. To learn more about the LymphoTrack Dx software, please refer to the LymphoTrack Dx Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
9-225-0009	LymphoTrack® Dx <i>TRB</i> Assay Kit A - MiSeq™	8 indices - 5 sequencing reactions each
9-225-0019	LymphoTrack® Dx <i>TRB</i> Assay Panel - MiSeq™	24 indices - 5 sequencing reactions each
9-500-0009	LymphoTrack® Dx Software - MiSeq™	1 CD complimentary with purchase

CE **IVD** These products are CE-IVD assays for *in vitro* diagnostic use and available in regions that accept CE-IVD products.

LymphoTrack[®] Dx

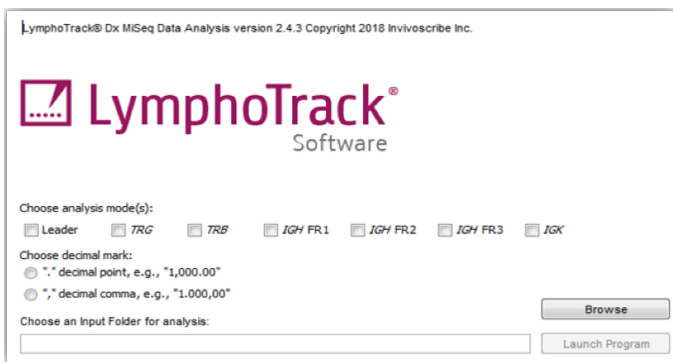
Bioinformatics Software

Software Use

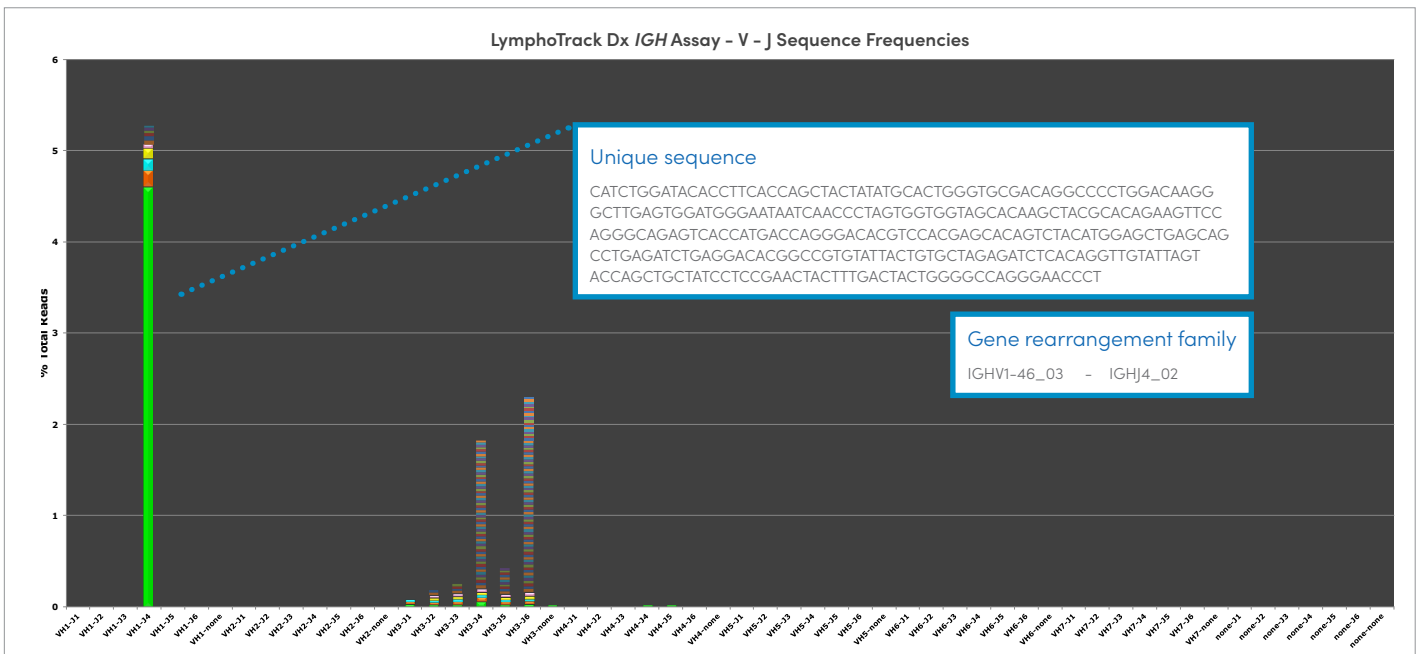
The LymphoTrack Dx Bioinformatics Software package is provided with each LymphoTrack Dx Assay to analyze raw FASTQ files for clonality analysis of single or multiple target data sets (*IGHV* Leader, *IGH* FR1, *IGH* FR2, *IGH* FR3, *IGK*, *TRB*, or *TRG*). For data generated with LymphoTrack Dx *IGHV* Leader or *IGH* FR1 Assays, the software provides additional information, including the rate of somatic hypermutation and whether a clone would be functional based upon the presence of a premature stop codon. The software can also predict whether an open reading frame would be in- or out-of-frame.

The provided software is composed of two distinct parts:

1. A bioinformatics Data Analysis Application
2. Microsoft Excel[®] Data Visualization spreadsheets and automated Sample-to-PDF Reports for streamlined data analysis for VJ usage and VJ sequence frequency graphs.



Sequence Frequency Graph



The stacked bar graph depicts the top 200 sequencing reads for a sample. Each individual colored bar represents a unique population of cells. Different colors stacked at the same point on the x-axis represent unique sequences that utilize the same V and J gene families. The amplicons of these products vary in sequence and may also vary in product size.

Sequencing Summary

Using the merged read summary, along with the easy to follow flow charts in the accompanying LymphoTrack Dx Assay instructions for use (IFU), interpretation is quick and easy.

Sample Name											
Total reads = 32,458											
Rank	Sequence	Length	Merge count	V-gene	J-gene	% Total reads	Cumulative %	Mutation rate partial V-gene (%)	In-frame (Y/N)	No stop codon (Y/N)	V-coverage
1	TTCTCGTGGTG	455	29603	IGHV4-59_08	IGHJ4_02	9.93	9.93	11.26	Y	Y	98.63
2	CTCGCCCTCCT	463	205	IGHV5-51_01	IGHJ4_02	0.07	9.99	0.00	Y	Y	99.66
3	GGTTTTCCTTG	484	201	IGHV3-7_01	IGHJ4_02	0.07	10.06	7.77	Y	Y	100.00
4	CTCGCCCTCCT	463	185	IGHV5-51_01	IGHJ5_02	0.06	10.12	6.08	Y	Y	99.32
5	CTCGCCCTCCT	469	170	IGHV5-51_01	IGHJ4_02	0.06	10.18	0.00	Y	Y	99.32
6	CTCGCCCTCCT	466	160	IGHV5-51_01	IGHJ4_02	0.05	10.23	0.00	Y	Y	99.66
7	CTGCTGCTGAC	460	159	IGHV2-5_10	IGHJ5_02	0.05	10.29	8.08	Y	Y	97.64
8	GGTTTTCCTTG	493	156	IGHV3-48_02	IGHJ6_02	0.05	10.34	3.72	Y	Y	98.99
9	CTCGCCCTCCT	334	153	IGHV5-51_02	IGHJ2_01	0.05	10.39	3.72	Y	N	27.70
10	CTCGCCCTCCT	334	152	IGHV5-51_02	IGHJ2_01	0.05	10.44	3.38	Y	N	26.01

- The sample name is clearly identified and the total number of reads (= Read Depth) generated for the sample is provided. Following the IFU, it is easy to determine whether the data generated for a sample can be assessed for the presence or absence of clonality.
- The sequence of clonal populations is provided and populations are ranked from most abundant to least prevalent. Sequences that differ by 1-2 basepairs are automatically merged to account for possible sequencing errors and to improve the accuracy and ease of sample interpretation.
- Sequences are aligned with reference genes to allow for easy identification of specific types of gene rearrangements such as *IGHV3-21*, which is characteristic of some CLL cases and correlates with a poor prognosis.
- The percentage that a unique sequence contributes to the total number of reads for a sample is calculated. Following the guidelines in the IFU, samples can be interpreted for the evidence indicating the presence or absence of clonality.
- For the LymphoTrack Dx *IGHV* Leader and *IGH* FR1 Assays, the somatic hypermutation status of a sequence is automatically calculated by comparing the identified sequence with a germline reference. In addition, predictions on whether the sequence would be functional can be drawn by the provided information regarding the presence of a premature stop codon or an open reading frame that is out-of-frame.

Ordering Information

Catalog #	Products	Quantity Components
9-500-0009	LymphoTrack® Dx Software - MiSeq™	Complimentary with LymphoTrack Dx Assay purchase
9-500-0007	LymphoTrack® Dx Software - S5/PGM™	Complimentary with LymphoTrack Dx Assay purchase

CE  These products are CE-IVD assays for *in vitro* diagnostic use and available in regions that accept CE-IVD products.



NEXT-GENERATION SEQUENCING (NGS) - RESEARCH USE ONLY (RUO)

Key Benefits

- » One-step PCR for amplicon and library generation
- » Identify and assess mutation status of B- and T-cell gene rearrangements
- » Sequence amplicons from any LymphoTrack kit together
- » Included bioinformatics software for easy analysis and interpretation
- » Same reagents for clonality, somatic hypermutation (SHM), minimal residual disease (MRD) testing, and tracking/monitoring of immunotherapy constructs

NGS Assays

- 42** LymphoTrack *IGHV* Leader Somatic Hypermutation Assay
- 44** LymphoTrack *IGH* FR1/2/3 Assays
- 48** LymphoTrack *IGK* Assays
- 50** LymphoTrack *TRG* Assays
- 52** LymphoTrack *TRB* Assay
- 54** LymphoTrack Bioinformatics Software

Warranty and Liability

Invivoscribe® (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe® shall have no liability for direct, indirect, consequential or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

Notice

This product is covered by one or more patents owned by or licensed to Invivoscribe, Inc., including US Patent No. 7,785,783, US Patent No. 8,859,748, US Patent No. 10,280,462 European Patent No. 1549764 (validated in 16 countries, and augmented by related European Patent Nos 2418287 and 2460889), European Patent No. 1633884, Japanese Patent No. 4708029, Japanese Patent No. 6189600, Brazilian Patent No. P10410283, Canadian Patent No. 2525122, Indian Patent No. 243620, Mexican Patent No. 286493, Chinese Patent No. 1806051, and Korean Patent No. 10-1215194.

These products use nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). Any necessary license to practice amplification methods or to use reagents, amplification enzymes or equipment covered by third party patents is the responsibility of the user and no such license is granted by Invivoscribe, Inc., expressly or by implication.



LymphoTrack Assay kits are designed for the identification of gene rearrangements in hematologic samples utilizing next-generation sequencing (NGS) technologies.

These assays take advantage of the wealth and depth of NGS data generated with either the Illumina® MiSeq™ or the Thermo Fisher Scientific® Ion PGM™ and Ion S5™ platforms. They offer significant improvements over conventional clonality testing methods, by providing the distribution of gene rearrangements, DNA sequences, the mutational status (*IGH* only), and the ability to track specific gene rearrangements all with the same workflow.

Primers included in the master mixes are designed with Illumina® adapters and indices (8 or 24 indices configurations for a total possible 24 or *48 unique indices [*FR1 only] per framework kits) or Thermo Fisher adapters and indices (12 indices per framework kits). By offering multiple kit configurations (8-or-24 indices for MiSeq™, 12 for Ion S5/PGM™), Invivoscribe provides laboratories the ability to choose the optimal kit for their sample throughput and read-depth requirements. Testing costs can be reduced by sequencing in a single run, with the possibility to combine: a) samples with up to 48 different indices and b) amplicons from other LymphoTrack Assays.

Disclaimer | Research Use Only (RUO) assays are not for sale in Europe and other global markets where equivalent CE-IVD assays are available and registered with the appropriate regulatory agencies. For more information regarding the CE-marked LymphoTrack® Dx Assays, please refer to the preceding pages.

IGHV Leader Somatic Hypermutation Assays

Assay Uses

This research use only (RUO) assay for next-generation sequencing (NGS), identifies clonal *IGH* V_H-J_H rearrangements, the associated V_H-J_H DNA sequences, and the frequency distribution of V_H region and J_H region segment utilization. The assay also uses the Illumina[®] MiSeq[™] platform to define the extent of somatic hypermutation (SHM) present in the *IGHV* gene of analyzed samples. If you would like to test for *IGHV* somatic hypermutation using the Thermo Fisher[®] Ion PGM[™] or Ion S5[™] platform please refer to the LymphoTrack *IGH* FR1 Assay (7-121-0007).

Summary and Explanation of the Test

The LymphoTrack *IGHV* Leader Somatic Hypermutation Assay for NGS provides significant improvements over clonality assays using fragment analysis and Sanger sequencing. The assay efficiently detects the majority of *IGH* gene rearrangements using a single multiplex master mix, identifies the DNA sequence specific for each clonal gene rearrangement, and calculates the degree of SHM for each sample.

The master mixes included in this assay target the Leader (V_HL) and the joining (J_H) gene regions of *IGH* and are designed with Illumina[®] adapters and indices (8 included in Kit A and 24 included in Panels). Including adapters and indices in the primer design allows for a one-step PCR approach to generate sequence-ready amplicons, followed by direct pooling of samples for sequencing on a Illumina[®] MiSeq[™] flow cell.

The included LymphoTrack bioinformatics software enables simplified analysis and visualization of data generated from this assay.

Positive (clonal positive, SHM negative), negative (polyclonal), and SHM positive (clonal positive, SHM positive) controls are included in the kit. Primers included in the master mixes are designed with Illumina[®] adapters and indices (8 or 24 indices per framework kits). This allows for a one-step PCR reaction to generate sequence-ready amplicons and pooling of several different samples on the same Illumina[®] MiSeq[™] flow cell. The LymphoTrack bioinformatics software enables easy analysis and visualization of data and the LymphoTrack MRD Software allows sequences to be tracked in subsequent samples. Please see the LymphoTrack MRD software section to learn how the LymphoTrack Assays can be applied to MRD studies, or email marketing@invivoscribe.com.

Background

The human immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.3) includes 46 - 52 functional and 30 nonfunctional variable (V_H) gene segments, 27 functional diversity (D_H) gene segments, and 6 functional joining (J_H) gene segments spread over 1250 kilobases.

During B-cell development, genes encoding the *IGH* molecules are assembled from multiple polymorphic gene segments that undergo rearrangements and selection, generating V_H-D_H-J_H combinations that are unique in both length and sequence.¹

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *IGH* clonal rearrangements can be useful in the study of B-cell malignancies. An additional level of diversity is generated in the antigen receptors by somatic point mutations in the variable regions and this mutation status provides important prognostic information for chronic lymphocytic leukemia (CLL)² and small lymphocytic lymphoma (SLL). In addition, NGS methods can improve disease stratification and elucidate subclone gene profiles.

Specimen Requirement

50 ng of high-quality genomic DNA.

References

1. Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 302.2.7.13 and 30.2.7.18.
2. Ghia et al., *Leukemia* 21: 2-3 (2007).



Simple representation of the organization of the immunoglobulin heavy chain (*IGH*) gene on chromosome 14. Depicted are the variable region (V_H) genes and downstream consensus joining region genes (J_H) that are involved in rearrangements. Upstream of the variable gene segments, the leader sequence (V_HL) is also depicted. Diversity region genes are not depicted.

Reagents - MiSeq™ Detection

Kit A Components		Panel Components (includes all master mixes from Kit A plus the items below)			
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
IGH Leader MiSeq 01	A001	IGH Leader MiSeq 09	A009	IGH Leader MiSeq 18	A018
IGH Leader MiSeq 02	A002	IGH Leader MiSeq 10	A010	IGH Leader MiSeq 19	A019
IGH Leader MiSeq 03	A003	IGH Leader MiSeq 11	A011	IGH Leader MiSeq 20	A020
IGH Leader MiSeq 04	A004	IGH Leader MiSeq 12	A012	IGH Leader MiSeq 21	A021
IGH Leader MiSeq 05	A005	IGH Leader MiSeq 13	A013	IGH Leader MiSeq 22	A022
IGH Leader MiSeq 06	A006	IGH Leader MiSeq 14	A014	IGH Leader MiSeq 23	A023
IGH Leader MiSeq 07	A007	IGH Leader MiSeq 15	A015	IGH Leader MiSeq 25	A025
IGH Leader MiSeq 08	A008	IGH Leader MiSeq 16	A016	IGH Leader MiSeq 27	A027
Controls		Controls			
IGH SHM POS (+) Qty. 1		IGH SHM POS (+) Qty. 3			
IGH POS (+) Qty. 1		IGH POS (+) Qty. 3			
NGS NEG (-) Qty. 1		NGS NEG (-) Qty. 3			

Rank	Sequence	Length	Merge count	V-gene	J-gene	% Total reads	Cumulative %	Mutation rate partial V-gene (%)	In-frame (Y/N)	No stop codon (Y/N)	V-coverage
1	TTCTCGTGGTG	455	29603	IGHV4-59_08	IGHJ4_02	9.93	9.93	11.26	Y	Y	98.63
2	CTCGCCCTCCT	463	205	IGHV5-51_01	IGHJ4_02	0.07	9.99	0.00	Y	Y	99.66
3	GGTTTTCTTG	484	201	IGHV3-7_01	IGHJ4_02	0.07	10.06	7.77	Y	Y	100.00
4	CTCGCCCTCCT	463	185	IGHV5-51_01	IGHJ5_02	0.06	10.12	6.08	Y	Y	99.32
5	CTCGCCCTCCT	469	170	IGHV5-51_01	IGHJ4_02	0.06	10.18	0.00	Y	Y	99.32
6	CTCGCCCTCCT	466	160	IGHV5-51_01	IGHJ4_02	0.05	10.23	0.00	Y	Y	99.66
7	CTGCTGCTGAC	460	159	IGHV2-5_10	IGHJ5_02	0.05	10.29	8.08	Y	Y	97.64
8	GGTTTTCTTG	493	156	IGHV3-48_02	IGHJ6_02	0.05	10.34	3.72	Y	Y	98.99
9	CTCGCCCTCCT	334	153	IGHV5-51_02	IGHJ2_01	0.05	10.39	3.72	Y	N	27.70
10	CTCGCCCTCCT	334	152	IGHV5-51_02	IGHJ2_01	0.05	10.44	3.38	Y	N	26.01

Example Data. Depicted are the top 10 sequences from a read summary generated by the LymphoTrack Software - MiSeq™. Highlighted columns represent fields that are unique to SHM analysis and include the SHM mutation rate and predictions pertaining to whether a sequence is in-frame or contains a premature stop codon. To learn more about the LymphoTrack software, please refer to the LymphoTrack Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
7-121-0059	LymphoTrack® IGHV Leader Somatic Hypermutation Assay Kit A - MiSeq™	8 indices - 5 sequencing reactions each
7-121-0069	LymphoTrack® IGHV Leader Somatic Hypermutation Assay Panel - MiSeq™	24 indices - 5 sequencing reactions each
7-500-0009	LymphoTrack® Software - MiSeq™	1 CD complimentary with purchase

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

IGH FR1/FR2/FR3 Assays

Assay Uses

These research use only (RUO) assays for next-generation sequencing (NGS), identify clonal *IGH* V_H-J_H rearrangements, the associated V_H-J_H region DNA sequences, the frequency distribution of V_H region and J_H region segment utilization. The LymphoTrack FR1 can also identify the degree of somatic hypermutation (SHM) of rearranged genes using the Illumina[®] MiSeq[™], Thermo Fisher Scientific[®] Ion PGM[™] or Ion S5[™]. The LymphoTrack *IGH* FR1, *IGH* FR2, and *IGH* FR3 Assays contain primers that target the conserved framework 1 (FR1), framework 2 (FR2), and framework 3 (FR3) regions, respectively. The LymphoTrack *IGH* FR1/2/3 Assay kits contain the master mixes of all three frameworks.

Summary and Explanation of the Test

The LymphoTrack *IGH* Assays represent a significant improvement over clonality assays that utilize fragment analysis by providing four important and complementary uses:

1. Detection of clonal populations.
2. Identification of sequence information and V_H-J_H segment utilization.
3. The LymphoTrack *IGH* FR1 Assays provide the degree of SHM of the immunoglobulin variable heavy chain (*IGHV*).
4. The ability to track sequences in subsequent samples with the Invivoscribe LymphoTrack MRD Software.

Each single multiplex master mix targets one of the conserved *IGH* framework regions (FR1, FR2, or FR3) within the V_H and the J_H regions described in lymphoid malignancies. **Targeting all three framework regions significantly reduces the risk of not being able to detect the presence of clonality**, as somatic hypermutations in the primer binding sites of the involved V_H gene segments can impede DNA amplification.¹

Primers included in the master mixes are designed with Illumina[®] adapters and indices (8 included in Kit A, 24 included in the Panel, and an independent 24 included in the Panel B) or Thermo Fisher adapters and indices (12 indices per framework kits). This allows for a one-step PCR reaction to generate sequence-ready amplicons and pooling of several different samples on the same Illumina[®] MiSeq[™] cell, Ion S5 or Ion PGM chip. The LymphoTrack bioinformatics software enables easy analysis and visualization of data and the LymphoTrack MRD Software allows sequences to be tracked in subsequent samples. Please see the LymphoTrack MRD software section to learn how the LymphoTrack Assays can be applied to MRD studies, or email marketing@invivoscribe.com.

Positive clonal (SHM negative) and negative polyclonal DNA controls are included in kits. A clonal SHM positive control can be purchased separately (4-088-0008).

Background

The human immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.3) includes 46-52 functional and 30 nonfunctional variable (V_H) gene segments, 27 functional diversity (D_H) gene segments, and 6 functional joining (J_H) gene segments spread over 1250 kilobases.

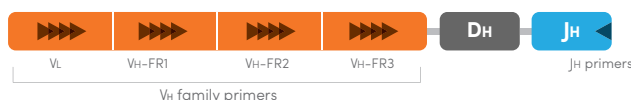
During development of lymphoid cells, the antigen receptor genes go through somatic gene rearrangements.¹ For example, during B-cell development, genes encoding the *IGH* molecules are assembled from multiple polymorphic gene segments that undergo rearrangements and selection, generating V_H-D_H-J_H combinations. Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *IGH* clonal populations can be useful in the study of B- and T-cell malignancies.

Specimen Requirement

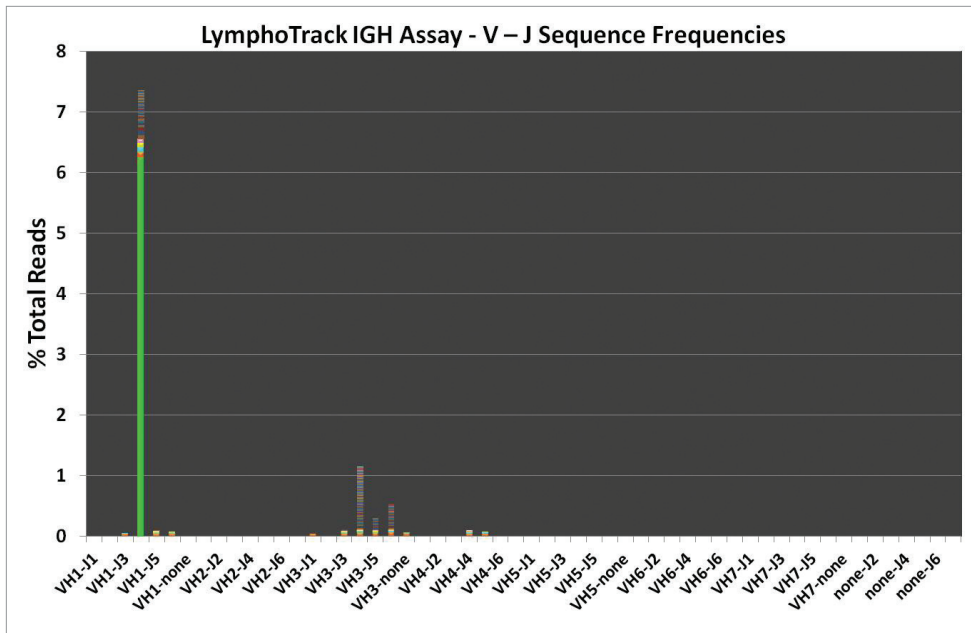
50 ng of high-quality genomic DNA.

Reference

1. S Tonegawa. *Nature* 302: 575-581 (1983).



Simple representation of the organization of the immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14. Depicted are the variable region (V_H) genes and downstream consensus joining region genes segments (J_H) that are involved in rearrangements.



V-J Sequence Frequencies. The LymphoTrack Software provides a stacked bar graph depicting the relative frequencies for the 200 most prevalent V_H - J_H rearrangements identified in a sample. To learn more about the LymphoTrack software, please refer to the LymphoTrack Bioinformatics Software section.

Ordering Information		
Catalog #	Products	Quantity Components
7-121-0129	LymphoTrack® IGH FR1/2/3 Assay Kit A - MiSeq™	Indices 1-8 (5 sequencing reactions each)
7-121-0139	LymphoTrack® IGH FR1/2/3 Assay Panel - MiSeq™	Indices 1-24 (5 sequencing reactions each)
7-121-0009	LymphoTrack® IGH FR1 Assay Kit A - MiSeq™	Indices 1-8 (5 sequencing reactions each)
7-121-0039	LymphoTrack® IGH FR1 Assay Panel - MiSeq™	Indices 1-24 (5 sequencing reactions each)
7-121-0149	LymphoTrack® IGH FR1 Assay Panel B - MiSeq™	Indices 25-48 (5 sequencing reaction each)
7-121-0089	LymphoTrack® IGH FR2 Assay Kit A - MiSeq™	Indices 1-8 (5 sequencing reactions each)
7-121-0099	LymphoTrack® IGH FR2 Assay Panel - MiSeq™	Indices 1-24 (5 sequencing reactions each)
7-121-0109	LymphoTrack® IGH FR3 Assay Kit A - MiSeq™	Indices 1-8 (5 sequencing reactions each)
7-121-0119	LymphoTrack® IGH FR3 Assay Panel - MiSeq™	Indices 1-24 (5 sequencing reactions each)
7-500-0009	LymphoTrack® Software - MiSeq™	1 CD complimentary with purchase
7-121-0057	LymphoTrack® IGH FR1/2/3 Assay - S5/PGM™	12 + 12 + 12 indices - 5 sequencing reactions each
7-121-0007	LymphoTrack® IGH FR1 Assay - S5/PGM™	12 indices - 5 sequencing reactions each
7-121-0037	LymphoTrack® IGH FR2 Assay - S5/PGM™	12 indices - 5 sequencing reactions each
7-121-0047	LymphoTrack® IGH FR3 Assay - S5/PGM™	12 indices - 5 sequencing reactions each
7-500-0007	LymphoTrack® Software - S5/PGM™	1 CD complimentary with purchase
7-500-0008	LymphoTrack® MRD Software	1 CD complimentary with purchase

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

IGH FR1/FR2/FR3 Assays Continued

Reagents - MiSeq™ Detection

The LymphoTrack IGH FR1/2/3 Assays contain components from respective individual FR Kit A's, Panel's or Panel B.

LymphoTrack IGH FR1 Components		LymphoTrack IGH FR2 Components		LymphoTrack IGH FR3 Components	
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
IGH FR1 MiSeq 01	A001	IGH FR2 MiSeq 01	A001	IGH FR3 MiSeq 01	A001
IGH FR1 MiSeq 02	A002	IGH FR2 MiSeq 02	A002	IGH FR3 MiSeq 02	A002
IGH FR1 MiSeq 03	A003	IGH FR2 MiSeq 03	A003	IGH FR3 MiSeq 03	A003
IGH FR1 MiSeq 04	A004	IGH FR2 MiSeq 04	A004	IGH FR3 MiSeq 04	A004
IGH FR1 MiSeq 05	A005	IGH FR2 MiSeq 05	A005	IGH FR3 MiSeq 05	A005
IGH FR1 MiSeq 06	A006	IGH FR2 MiSeq 06	A006	IGH FR3 MiSeq 06	A006
IGH FR1 MiSeq 07	A007	IGH FR2 MiSeq 07	A007	IGH FR3 MiSeq 07	A007
IGH FR1 MiSeq 08	A008	IGH FR2 MiSeq 08	A008	IGH FR3 MiSeq 08	A008
IGH FR1 MiSeq 09	A009	IGH FR2 MiSeq 09	A009	IGH FR3 MiSeq 09	A009
IGH FR1 MiSeq 10	A010	IGH FR2 MiSeq 10	A010	IGH FR3 MiSeq 10	A010
IGH FR1 MiSeq 11	A011	IGH FR2 MiSeq 11	A011	IGH FR3 MiSeq 11	A011
IGH FR1 MiSeq 12	A012	IGH FR2 MiSeq 12	A012	IGH FR3 MiSeq 12	A012
IGH FR1 MiSeq 13	A013	IGH FR2 MiSeq 13	A013	IGH FR3 MiSeq 13	A013
IGH FR1 MiSeq 14	A014	IGH FR2 MiSeq 14	A014	IGH FR3 MiSeq 14	A014
IGH FR1 MiSeq 15	A015	IGH FR2 MiSeq 15	A015	IGH FR3 MiSeq 15	A015
IGH FR1 MiSeq 16	A016	IGH FR2 MiSeq 16	A016	IGH FR3 MiSeq 16	A016
IGH FR1 MiSeq 18	A018	IGH FR2 MiSeq 18	A018	IGH FR3 MiSeq 18	A018
IGH FR1 MiSeq 19	A019	IGH FR2 MiSeq 19	A019	IGH FR3 MiSeq 19	A019
IGH FR1 MiSeq 20	A020	IGH FR2 MiSeq 20	A020	IGH FR3 MiSeq 20	A020
IGH FR1 MiSeq 21	A021	IGH FR2 MiSeq 21	A021	IGH FR3 MiSeq 21	A021
IGH FR1 MiSeq 22	A022	IGH FR2 MiSeq 22	A022	IGH FR3 MiSeq 22	A022
IGH FR1 MiSeq 23	A023	IGH FR2 MiSeq 23	A023	IGH FR3 MiSeq 23	A023
IGH FR1 MiSeq 25	A025	IGH FR2 MiSeq 25	A025	IGH FR3 MiSeq 25	A025
IGH FR1 MiSeq 27	A027	IGH FR2 MiSeq 27	A027	IGH FR3 MiSeq 27	A027
IGH FR1 MiSeq 17	A017				
IGH FR1 MiSeq 24	A024				
IGH FR1 MiSeq 26	A026				
IGH FR1 MiSeq 28	A028				
IGH FR1 MiSeq 29	A029				
IGH FR1 MiSeq 30	A030				
IGH FR1 MiSeq 31	A031				
IGH FR1 MiSeq 32	A032				
IGH FR1 MiSeq 33	A033				
IGH FR1 MiSeq 34	A034				
IGH FR1 MiSeq 35	A035				
IGH FR1 MiSeq 36	A036				
IGH FR1 MiSeq 37	A037				
IGH FR1 MiSeq 38	A038				
IGH FR1 MiSeq 39	A039				
IGH FR1 MiSeq 40	A040				

Reagents – MiSeq™ Detection cont.

The LymphoTrack *IGH* FR1/2/3 Assays contain components from respective individual FR Kit A's, Panel's or Panel B.

LymphoTrack <i>IGH</i> FR1 Components		LymphoTrack <i>IGH</i> FR2 Components		LymphoTrack <i>IGH</i> FR3 Components	
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
<i>IGH</i> FR1 MiSeq 41	A041				
<i>IGH</i> FR1 MiSeq 42	A042				
<i>IGH</i> FR1 MiSeq 43	A043				
<i>IGH</i> FR1 MiSeq 44	A044				
<i>IGH</i> FR1 MiSeq 45	A045				
<i>IGH</i> FR1 MiSeq 46	A046				
<i>IGH</i> FR1 MiSeq 47	A047				
<i>IGH</i> FR1 MiSeq 48	A048				
<i>IGH</i> FR1 MiSeq 49	A049				

PANEL B

Controls in Individual FR (1, 2, or 3) Kit A's	Controls in Individual FR (1,2, or 3) Panels	Controls in Combo FR 1/2/3 Kit A	Controls in Combo FR 1/2/3 Panel
<i>IGH</i> POS (+) Qty. 1	<i>IGH</i> POS (+) Qty. 3	<i>IGH</i> POS (+) Qty. 2	<i>IGH</i> POS (+) Qty. 6
NGS NEG (-) Qty. 1	NGS NEG (-) Qty. 3	NGS NEG (-) Qty. 2	NGS NEG (-) Qty. 6

Reagents – Ion S5™/PGM™ Detection

The LymphoTrack *IGH* FR1/2/3 Assays contain components from respective individual FR Assays.

LymphoTrack <i>IGH</i> FR1 Components		LymphoTrack <i>IGH</i> FR2 Components		LymphoTrack <i>IGH</i> FR3 Components	
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
<i>IGH</i> FR1 S5/PGM 01	IonXpress_001	<i>IGH</i> FR2 S5/PGM 01	IonXpress_001	<i>IGH</i> FR3 S5/PGM 01	IonXpress_001
<i>IGH</i> FR1 S5/PGM 02	IonXpress_002	<i>IGH</i> FR2 S5/PGM 02	IonXpress_002	<i>IGH</i> FR3 S5/PGM 02	IonXpress_002
<i>IGH</i> FR1 S5/PGM 03	IonXpress_003	<i>IGH</i> FR2 S5/PGM 03	IonXpress_003	<i>IGH</i> FR3 S5/PGM 03	IonXpress_003
<i>IGH</i> FR1 S5/PGM 04	IonXpress_004	<i>IGH</i> FR2 S5/PGM 04	IonXpress_004	<i>IGH</i> FR3 S5/PGM 04	IonXpress_004
<i>IGH</i> FR1 S5/PGM 07	IonXpress_007	<i>IGH</i> FR2 S5/PGM 07	IonXpress_007	<i>IGH</i> FR3 S5/PGM 07	IonXpress_007
<i>IGH</i> FR1 S5/PGM 08	IonXpress_008	<i>IGH</i> FR2 S5/PGM 08	IonXpress_008	<i>IGH</i> FR3 S5/PGM 08	IonXpress_008
<i>IGH</i> FR1 S5/PGM 09	IonXpress_009	<i>IGH</i> FR2 S5/PGM 09	IonXpress_009	<i>IGH</i> FR3 S5/PGM 09	IonXpress_009
<i>IGH</i> FR1 S5/PGM 10	IonXpress_010	<i>IGH</i> FR2 S5/PGM 10	IonXpress_010	<i>IGH</i> FR3 S5/PGM 10	IonXpress_010
<i>IGH</i> FR1 S5/PGM 11	IonXpress_011	<i>IGH</i> FR2 S5/PGM 11	IonXpress_011	<i>IGH</i> FR3 S5/PGM 11	IonXpress_011
<i>IGH</i> FR1 S5/PGM 12	IonXpress_012	<i>IGH</i> FR2 S5/PGM 12	IonXpress_012	<i>IGH</i> FR3 S5/PGM 12	IonXpress_012
<i>IGH</i> FR1 S5/PGM 13	IonXpress_013	<i>IGH</i> FR2 S5/PGM 13	IonXpress_013	<i>IGH</i> FR3 S5/PGM 13	IonXpress_013
<i>IGH</i> FR1 S5/PGM 14	IonXpress_014	<i>IGH</i> FR2 S5/PGM 14	IonXpress_014	<i>IGH</i> FR3 S5/PGM 14	IonXpress_014

Controls in Individual FR (1,2, or 3) Kits	Controls in FR 1/2/3 Kit
<i>IGH</i> POS (+) Qty. 2	<i>IGH</i> POS (+) Qty. 4
NGS NEG (-) Qty. 2	NGS NEG (-) Qty. 4

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

LymphoTrack®

IGK Assay

Assay Uses

This research use only (RUO) assay for next-generation sequencing (NGS), identifies clonal *IGK* V_k-J_k, V_k-K_{de}, and intron-K_{de} (INTR-K_{de}) rearrangements, the corresponding DNA sequences, and provides the distribution frequency of V_k-J_k, V_k-K_{de}, and INTR-K_{de} segment utilization using the Illumina® MiSeq™ or Thermo Fisher Scientific® Ion PGM™ and Ion S5™ platforms.

Summary and Explanation of the Test

The LymphoTrack *IGK* Assay represents a significant improvement over clonality assays that utilize fragment analysis by providing three important and complementary uses:

1. Detection of clonal populations.
2. Identification of sequence information and gene segment utilization.
3. Ability to track sequences in subsequent samples with the use of the LymphoTrack MRD Software.

Unlike conventional fragment analysis assays, this NGS method utilizes a single multiplex master mix to target conserved regions of *IGK* that are described in lymphoid malignancies. Primers are designed with Illumina® adapters and indices (8-24) or Thermo Fisher Scientific adapters and indices (12), thereby allowing for a one-step PCR reaction to generate sequence-ready amplicons. To reduce per sample testing costs, amplicons from different samples (amplified with different indexed master mixes) or LymphoTrack kits can be sequenced together on a single Illumina® MiSeq™ flow cell, Ion S5™ or PGM™ chips.

The LymphoTrack bioinformatics software enables simplified analysis and visualization of data and the LymphoTrack MRD Software allows identified sequences to be tracked in subsequent samples. Please see the LymphoTrack MRD software section to learn how the LymphoTrack Assays can be applied to MRD studies, or email marketing@invivoscribe.com. Positive (clonal) and negative (polyclonal) DNA controls are included in the kits.

Background

The human immunoglobulin kappa (*IGK*) gene locus on chromosome 2 (2p11.2) includes 76 V (variable) region genes spanning 7 subgroups and 5 J (joining) region gene segments upstream of the C_k region. The K_{de}, approximately 24 kb downstream of the J_k-C_k region, can also rearrange with V_k gene segments and the isolated recombination signal sequence in the J_k-C_k intronic region.

During development of lymphoid cells, antigen receptor genes undergo somatic gene rearrangements.¹ Specifically during B-cell development, genes encoding *IGK* molecules are assembled from multiple polymorphic gene segments that undergo rearrangements generating V-J combinations unique in both length and sequence.² Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *IGK* clonal populations can be useful in the study of B-cell malignancies and complement *IGH* testing, as the *IGK* receptor is less susceptible to somatic mutations.

Specimen Requirement

50 ng of high-quality genomic DNA.

References

1. S Tonegawa et al., *Nature* 302: 575-581 (1983).
2. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 30.2.7.13 and 30.2.7.18.

Simplified Representation of the *IGK* Gene

Depicted are the variable region (V_k) genes or variable intragenic J_k-C_k intron (J_k-C_k INTR) and downstream consensus joining region genes (J_k) or kappa deleting element (K_{de}) that are involved in *IGK* gene rearrangements.

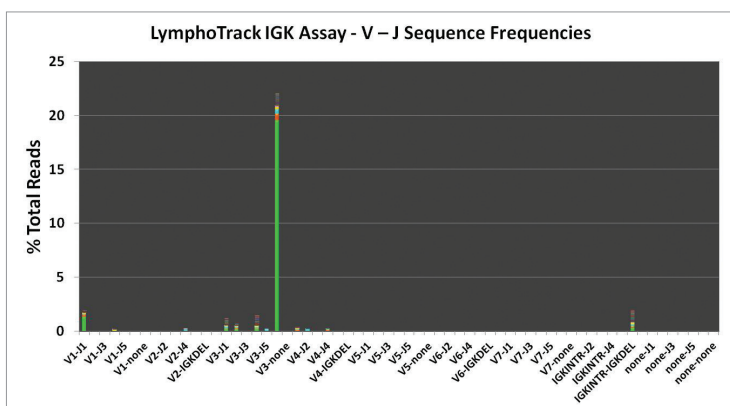


Reagents - MiSeq™ Detection

Kit A Components		Panel Components (includes all master mixes from Kit A plus the items below)			
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
IGK MiSeq 01	A001	IGK MiSeq 09	A009	IGK MiSeq 18	A018
IGK MiSeq 02	A002	IGK MiSeq 10	A010	IGK MiSeq 19	A019
IGK MiSeq 03	A003	IGK MiSeq 11	A011	IGK MiSeq 20	A020
IGK MiSeq 04	A004	IGK MiSeq 12	A012	IGK MiSeq 21	A021
IGK MiSeq 05	A005	IGK MiSeq 13	A013	IGK MiSeq 22	A022
IGK MiSeq 06	A006	IGK MiSeq 14	A014	IGK MiSeq 23	A023
IGK MiSeq 07	A007	IGK MiSeq 15	A015	IGK MiSeq 25	A025
IGK MiSeq 08	A008	IGK MiSeq 16	A016	IGK MiSeq 27	A027
Controls		Controls			
IGK POS (+) Qty. 1	NGS NEG (-) Qty. 1	IGK POS (+) Qty. 3		NGS NEG (-) Qty. 3	

Reagents - Ion S5/PGM™ Detection

Assay Components			
Master Mix Name	Index #	Master Mix Name	Index #
IGK S5/PGM 01	IonXpress_001	IGK S5/PGM 11	IonXpress_011
IGK S5/PGM 02	IonXpress_002	IGK S5/PGM 12	IonXpress_012
IGK S5/PGM 04	IonXpress_004	IGK S5/PGM 13	IonXpress_013
IGK S5/PGM 08	IonXpress_008	IGK S5/PGM 14	IonXpress_014
IGK S5/PGM 09	IonXpress_009	IGK S5/PGM 16	IonXpress_016
IGK S5/PGM 010	IonXpress_010	IGK S5/PGM 17	IonXpress_017
Controls			
IGK POS (+) Qty. 2		NGS NEG (-) Qty. 2	



V-J Sequence Frequencies.

The LymphoTrack bioinformatics software provides a stacked bar graph depicting the relative frequencies for the 200 most prevalent rearrangements sequenced and identified in the sample. To learn more about the LymphoTrack software, please refer to the LymphoTrack Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
7-122-0009	LymphoTrack® IGK Assay Kit A - MiSeq™	8 indices - 5 sequencing reactions each
7-122-0019	LymphoTrack® IGK Assay Panel - MiSeq™	24 indices - 5 sequencing reactions each
7-500-0009	LymphoTrack® Software - MiSeq™	1 CD complimentary with purchase
7-122-0007	LymphoTrack® IGK Assay - S5/PGM™	12 indices - 5 sequencing reactions each
7-500-0007	LymphoTrack® Software - S5/PGM™	1 CD complimentary with purchase
7-500-0008	LymphoTrack® MRD Software*	1 CD complimentary with purchase

*MRD Software can be used to track sequences generated by either LymphoTrack Assays - MiSeq™ or Ion S5/PGM™. These products are for Research Use Only (RUO). Not intended for diagnostic purposes.



TRG Assay

Assay Uses

This research use only (RUO) assay for next-generation sequencing (NGS) identifies clonal *TRG* V-J rearrangements and the associated V-J region DNA sequences. It also provides the frequency distribution of V-J segment utilization using the Illumina® MiSeq™ or Thermo Fisher Scientific® Ion S5™ and PGM™ instruments.

Summary and Explanation of the Test

The LymphoTrack *TRG* Assay represents a significant improvement over existing clonality assays that utilize fragment analysis by providing three important and complementary uses:

1. Detection of clonal populations.
2. Identification of sequence information and gene segment utilization.
3. Ability to track sequences in subsequent samples with the use of the Invivoscribe MRD Software.

This assay utilizes a single multiplex master mix to target conserved V and J regions of the human *TRB* gene locus described in lymphoid malignancies, thereby reducing sample DNA requirements and simplifying the testing workflow. Primers are designed with Illumina® adapters and indices (8–24) or Thermo Fisher Scientific adapters and indices (12), thereby allowing for a one-step PCR reaction to generate sequence-ready amplicons. In addition, amplicons from different samples (amplified with different indexed master mixes) or LymphoTrack kits can be sequenced together on a single Illumina® MiSeq™ flow cell Ion S5™ or PGM™ chip to reduce per sample testing costs.

The LymphoTrack bioinformatics software enables simplified analysis and visualization of data generated from this assay and the LymphoTrack MRD Software allows sequences to be tracked in subsequent samples. Please see the LymphoTrack MRD software section to learn how the LymphoTrack Assays can be applied to MRD studies, or email marketing@invivoscribe.com.

Positive (clonal) and negative (polyclonal) DNA controls are included in the kits.

Background

The human T-cell receptor gamma (*TRG*) gene locus on chromosome 7 (7q14) includes 14 variable region (Group I, II, III, and IV), 5 joining region (J) gene segments, and 2 constant (C) genes spread over 200 kilobases.

During development of lymphoid cells, the antigen receptor genes (including gene segments within the *TRG* locus), undergo somatic gene rearrangement.¹ These developmentally regulated gene rearrangements generate V–J combinations that are unique for each cell.² Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or “clonal” antigen receptor gene rearrangements. Therefore, tests that detect *TRG* clonal populations can be useful in the study of T-cell and certain B-cell malignancies. Since the *TRG* locus rearranges before the *TRB* locus, and all unsuccessful or unproductive rearrangements are retained in the cells, examination of the *TRG* locus can identify both clonal Δ/γ as well as clonal α/β T-cells. Clonal α/β T-cells would be expected to have biallelic *TRG* gene rearrangements.

Note | For a more thorough explanation of the locus and the targeted deep sequencing strategy, please refer to Principle of Immunoglobulin and T-Cell Receptor Gene Rearrangement.²

Specimen Requirement

50 ng of high-quality genomic DNA.

References

1. Tonegawa, S. (1983). *Nature* 302, 575–581.
2. Miller JE. (2013) *Molecular Genetic Pathology* (2nd Edition, sections 30.2.7.13 and 30.2.7.18).

Simplified Representation of the *TRG* Gene



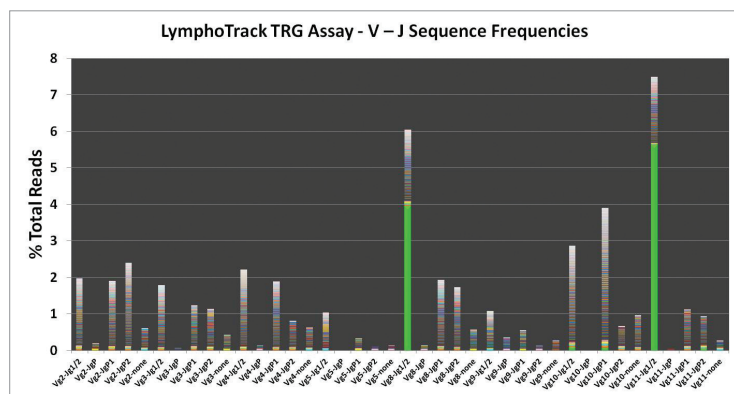
Simple representation of the organization of the T-cell receptor gamma gene on chromosome 7. Depicted are the variable region genes (V γ 2–V γ 11) and downstream joining region genes (J γ 1/2–J γ P1/2) that are involved in rearrangements in T-cell lymphomas.

Reagents - MiSeq™ Detection

Kit A Components		Panel Components (includes all master mixes from Kit A plus the items below)			
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
TRG MiSeq 01	A001	TRG MiSeq 09	A009	TRG MiSeq 18	A018
TRG MiSeq 02	A002	TRG MiSeq 10	A010	TRG MiSeq 19	A019
TRG MiSeq 03	A003	TRG MiSeq 11	A011	TRG MiSeq 20	A020
TRG MiSeq 04	A004	TRG MiSeq 12	A012	TRG MiSeq 21	A021
TRG MiSeq 05	A005	TRG MiSeq 13	A013	TRG MiSeq 22	A022
TRG MiSeq 06	A006	TRG MiSeq 14	A014	TRG MiSeq 23	A023
TRG MiSeq 07	A007	TRG MiSeq 15	A015	TRG MiSeq 25	A025
TRG MiSeq 08	A008	TRG MiSeq 16	A016	TRG MiSeq 27	A027
Controls		Controls			
TRG POS (+) Qty. 1	NGS NEG (-) Qty. 1	TRG POS (+) Qty. 3			NGS NEG (-) Qty. 3

Reagents - Ion S5/PGM™ Detection

Assay Components					
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
TRG S5/PGM 01	IonXpress_001	TRG S5/PGM 07	IonXpress_007	TRG S5/PGM 11	IonXpress_011
TRG S5/PGM 02	IonXpress_002	TRG S5/PGM 08	IonXpress_008	TRG S5/PGM 12	IonXpress_012
TRG S5/PGM 03	IonXpress_003	TRG S5/PGM 09	IonXpress_009	TRG S5/PGM 13	IonXpress_013
TRG S5/PGM 04	IonXpress_004	TRG S5/PGM 10	IonXpress_010	TRG S5/PGM 14	IonXpress_014
Controls					
TRG POS (+) Qty. 2			NGS NEG (-) Qty. 2		



V - J Sequence Frequencies.

The LymphoTrack bioinformatics software provides a stacked bar graph depicting the relative frequencies for the V-J rearrangements identified and sequenced in a sample. To learn more about the LymphoTrack software, please refer to the LymphoTrack Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
7-227-0019	LymphoTrack® TRG Assay Kit A - MiSeq™	8 indices - 5 sequencing reactions each
7-227-0009	LymphoTrack® TRG Assay Panel - MiSeq™	24 indices - 5 sequencing reactions each
7-500-0009	LymphoTrack® Software - MiSeq™	1 CD complimentary with purchase
7-227-0007	LymphoTrack® TRG Assay - S5/PGM™	12 indices - 5 sequencing reactions each
7-500-0007	LymphoTrack® Software - S5/PGM™	1 CD complimentary with purchase
7-500-0008	LymphoTrack® MRD Software*	1 CD complimentary with purchase

*MRD Software can be used to track sequences generated by either LymphoTrack Assays - MiSeq™ or Ion S5/PGM™. These products are for Research Use Only (RUO). Not intended for diagnostic purposes.



TRB Assay

Assay Uses

This research use only (RUO) assay for next-generation sequencing (NGS) identifies clonal *TRB* V β -(D β -)J β rearrangements, the associated V β -(D β -)J β region DNA sequences, and provides the frequency distribution of V β , D β , and J β region segment utilization using the Illumina® MiSeq™ platform.

Analysis of the rearranged *TRB* locus increases the probability of identifying T-cell receptor gene rearrangements, as compared to testing for *TRG* gene rearrangements only. As a result, combining the analysis of *TRB* and *TRG* loci increases the sensitivity of clonality detection.

Summary and Explanation of the Test

This assay utilizes a single multiplex master mix to target conserved V, D and J regions of the human *TRB* gene locus described in lymphoid malignancies, thereby reducing sample DNA requirements and simplifying the testing workflow.

The LymphoTrack *TRB* master mix primers are also designed with Illumina® adapters and 8 indices (Kit A) or 24 indices (Panel). This allows amplicons generated from different indexed *TRB* master mixes to be pooled into a single sequencing library.

The associated LymphoTrack Software is capable of sorting complex NGS data by gene target. This offers a second layer of multiplexing to reduce per sample testing costs by allowing amplicons from any LymphoTrack Assay (e.g. *IGH*, *IGK*, *TRB*, *TRG*) to be sequenced on the same flow cell. In addition, the LymphoTrack Software provides easy visualization of data and the LymphoTrack MRD Software allows identified sequences to be tracked and monitored in subsequent samples.

Positive clonal and negative polyclonal DNA controls are included in kits. Please see the LymphoTrack MRD software section to learn how the LymphoTrack Assays can be applied to MRD studies, or email marketing@invivoscribe.com.

Background

The LymphoTrack *TRB* Assay represent a significant improvement over clonality assessment by fragment analysis by providing two important and complementary uses:

1. Detection of clonal populations.
2. Identification of sequence information and gene segment utilization.
3. Ability to track sequences in subsequent samples with the use of the Invivoscribe MRD Software.

The human T-cell receptor beta (*TRB*) gene locus on chromosome 7 (7q34) includes 65 V β (variable) gene segments, followed by two separate clusters of genes each containing a D β (diversity) gene, several J β (joining) genes, and a C β (constant) region spread over 685 kilobases. The two C β genes, *TRBC1* and *TRBC2*, encode highly homologous products with no functional difference.

During lymphoid cell development, antigen receptor genes undergo somatic gene rearrangements.¹ Specifically, during T-cell development, genes encoding *TRB* molecules are assembled from multiple polymorphic gene segments that generate V β -D β -J β combinations unique in both length and sequence.²

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *TRB* clonal rearrangements can be useful in the study of B- and T-cell malignancies.

Note: For a more thorough explanation of the locus and the targeted deep sequencing strategy, please refer to Principle of Immunoglobulin and T-Cell Receptor Gene Rearrangement.²

Specimen Requirement

50 ng of high-quality genomic DNA.

References

1. Tonegawa, S. (1983). *Nature* 302, 575-581.
2. Miller JE. (2013) *Molecular Genetic Pathology* (2nd Edition, sections 30.2.7.13 and 30.2.7.18).

Simplified Representation of the *TRB* Gene

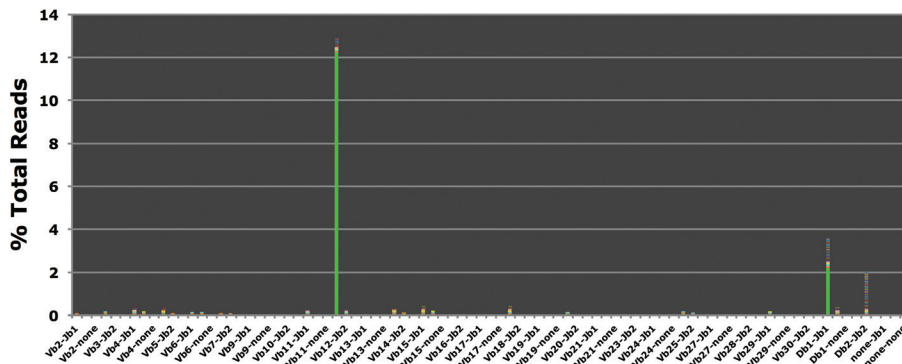


Depicted are the variable (V β), diversity (D β), and joining (J β) gene regions involved in *TRB* gene rearrangements, in addition to the downstream consensus (C β) gene regions.

Reagents - MiSeq™ Detection

Kit A Components		Panel Components (includes all master mixes from Kit A plus the items below)			
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
<i>TRB</i> MiSeq 01	A001	<i>TRB</i> MiSeq 09	A009	<i>TRB</i> MiSeq 18	A018
<i>TRB</i> MiSeq 02	A002	<i>TRB</i> MiSeq 10	A010	<i>TRB</i> MiSeq 19	A019
<i>TRB</i> MiSeq 03	A003	<i>TRB</i> MiSeq 11	A011	<i>TRB</i> MiSeq 20	A020
<i>TRB</i> MiSeq 04	A004	<i>TRB</i> MiSeq 12	A012	<i>TRB</i> MiSeq 21	A021
<i>TRB</i> MiSeq 05	A005	<i>TRB</i> MiSeq 13	A013	<i>TRB</i> MiSeq 22	A022
<i>TRB</i> MiSeq 06	A006	<i>TRB</i> MiSeq 14	A014	<i>TRB</i> MiSeq 23	A023
<i>TRB</i> MiSeq 07	A007	<i>TRB</i> MiSeq 15	A015	<i>TRB</i> MiSeq 25	A025
<i>TRB</i> MiSeq 08	A008	<i>TRB</i> MiSeq 16	A016	<i>TRB</i> MiSeq 27	A027
Controls		Controls			
<i>TRB</i> POS (+) Qty. 1	NGS NEG (-) Qty. 1	<i>TRB</i> POS (+) Qty. 3		NGS NEG (-) Qty. 3	

LymphoTrack *TRB* Assay - V - J Sequence Frequencies



V-J Sequence Frequencies.

The LymphoTrack bioinformatics software provides PDF reports which include Top 10 Merged Read Summary as well as a stacked bar graph depicting the relative frequencies of the 200 most prevalent rearrangements sequenced and identified in the sample. To learn more about the LymphoTrack software, please refer to the LymphoTrack Bioinformatics Software section.

Ordering Information

Catalog #	Products	Quantity Components
7-225-0009	LymphoTrack® <i>TRB</i> Assay Kit A - MiSeq™	8 indices - 5 sequencing reactions each
7-225-0019	LymphoTrack® <i>TRB</i> Assay Panel - MiSeq™	24 indices - 5 sequencing reactions each
7-500-0009	LymphoTrack® Software - MiSeq™	1 CD complimentary with purchase
7-500-0008	LymphoTrack® MRD Software*	1 CD complimentary with purchase

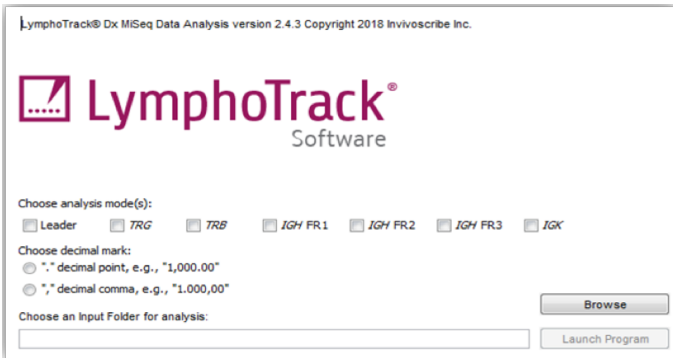
These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

Software Use

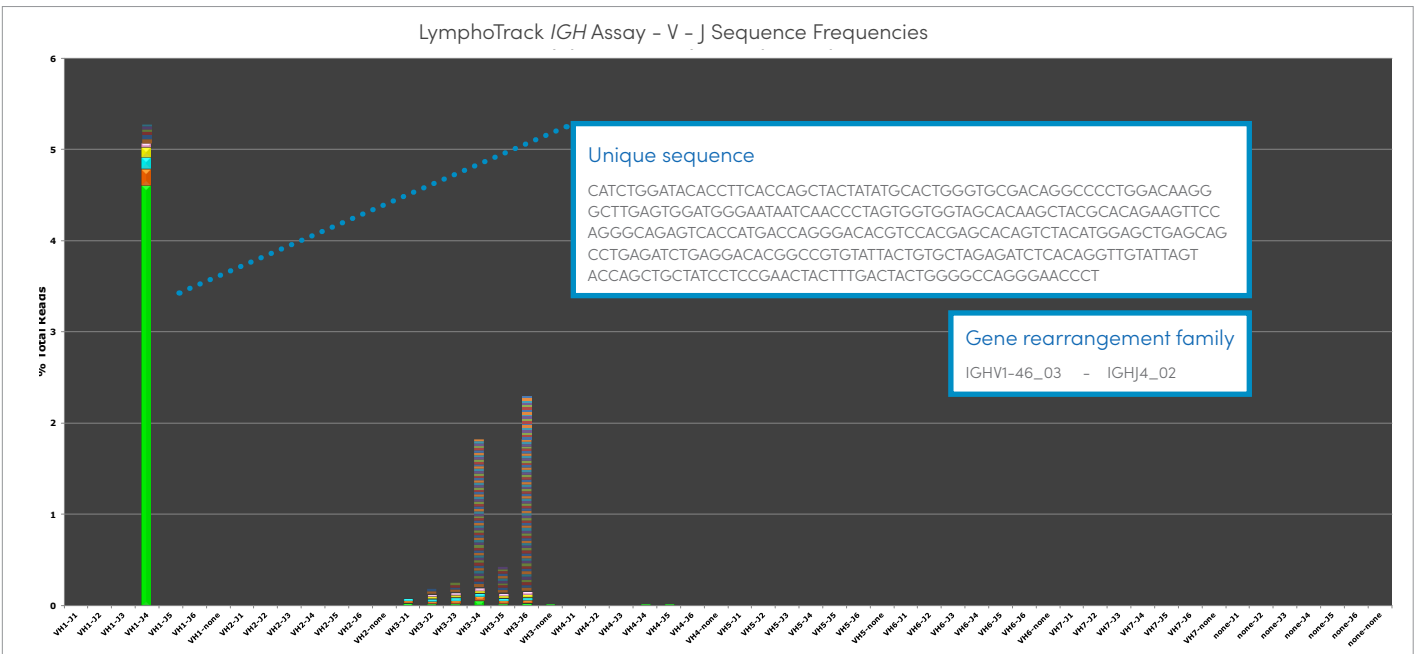
The LymphoTrack Bioinformatics Software package is provided with each LymphoTrack Assay to analyze raw FASTQ files for clonality analysis of single or multiple target data sets (*IGHV* Leader), *IGH* FR1, *IGH* FR2, *IGH* FR3, *IGK*, *TRG*, *TRB*). For data generated with LymphoTrack *IGHV* Leader or *IGH* FR1 Assays the software provides additional information, including the rate of somatic hypermutation (SHM) and whether a clone will be functional based upon the presence of a premature stop codon. The software can also predict whether an open reading frame would be in- or out-of-frame, so no external data analysis is required for sample interpretation.

The provided software is composed of two distinct parts:

1. A bioinformatics Data Analysis Application
2. Microsoft Excel® Data Visualization spreadsheets for each gene target and automated Sample-to-PDF Reports for streamlined data analysis.



Sequence Frequency Graph



The stacked bar graph depicts the top 200 sequencing reads for a sample. Each individual colored bar represents a unique population of cells. Different colors stacked at the same point on the x-axis represent unique sequences that utilize the same V and J gene families. The amplicons of these products vary in sequence and may also vary in product size.

Sequencing Summary

Using the merged read summary interpretation is quick and easy.

1 Easy identification of specific types of gene rearrangements such as *IGHV3-21*.

Rank	Sequence	Length	Merge count	V-gene	J-gene	% Total reads	Cumulative %	Mutation rate partial V-gene (%)	In-frame (Y/N)	No stop codon (Y/N)	V-coverage
1	TTCTCGTGGTG	455	29603	IGHV4-59_08	IGHJ4_02	9.93	9.93	11.26	Y	Y	98.63
2	CTCGCCCTCCT	463	205	IGHV5-51_01	IGHJ4_02	0.07	9.99	0.00	Y	Y	99.66
3	GGTTTTCCTTG	484	201	IGHV3-7_01	IGHJ4_02	0.07	10.06	7.77	Y	Y	100.00
4	CTCGCCCTCCT	463	185	IGHV5-51_01	IGHJ5_02	0.06	10.12	6.08	Y	Y	99.32
5	CTCGCCCTCCT	469	170	IGHV5-51_01	IGHJ4_02	0.06	10.18	0.00	Y	Y	99.32
6	CTCGCCCTCCT	466	160	IGHV5-51_01	IGHJ4_02	0.05	10.23	0.00	Y	Y	99.66
7	CTGCTGCTGAC	460	159	IGHV2-5_10	IGHJ5_02	0.05	10.29	8.08	Y	Y	97.64
8	GGTTTTCCTTG	493	156	IGHV3-48_02	IGHJ6_02	0.05	10.34	3.72	Y	Y	98.99
9	CTCGCCCTCCT	334	153	IGHV5-51_02	IGHJ2_01	0.05	10.39	3.72	Y	N	27.70
10	CTCGCCCTCCT	334	152	IGHV5-51_02	IGHJ2_01	0.05	10.44	3.38	Y	N	26.01

2 Identification of clonal sequences for follow up tracking with LymphoTrack MRD Software.

3 SHM rate and indicators to determine whether a clone is productive. Only provided for *IGHV* Leader and *IGH* FR1.

The read summary provides sequences from a sample ranked from most abundant to least prevalent. The total read count for individual sequences is provided and no independent analysis is required to determine V and J gene families and predictions for SHM when analyzing data from LymphoTrack *IGHV* Leader or *IGH* FR1 Assays. Additionally, the software provides raw and merged data in which reads that differ by 1-2 bp are automatically merged to account for possible sequencing errors and to improve the accuracy and ease of sample interpretation. combined.

Ordering Information

Catalog #	Products	Quantity Components
7-500-0009	LymphoTrack® Software- MiSeq™	Complimentary with LymphoTrack Assay purchase
7-500-0007	LymphoTrack® Software - S5/PGM™	Complimentary with LymphoTrack Assay purchase

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.



MINIMAL RESIDUAL DISEASE (MRD) SOLUTION – RESEARCH USE ONLY (RUO)

Key Benefits

- » Complete solution for MRD clonality
- » Ensures test sensitivity to enable confidence in reporting
- » Facilitates standardization of clonotype tracking
- » LymphoTrack MRD Software for automated analyses with PDF Reports
- » Longitudinal assessment of mutation status of *IGH* and T-cell clonality including gene rearrangements and somatic hypermutation (SHM)
- » LymphoTrack Assays formatted for both Illumina® and Thermo Fisher® NGS Platforms available

MRD Assays

58 LymphoTrack Minimal Residual Disease (MRD) Software

60 LymphoTrack B-cell & T-cell Low Positive Controls

61 LymphoQuant B-cell & T-cell Internal Control

Warranty and Liability

Invivoscribe® (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe® shall have no liability for direct, indirect, consequential or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

Notice

This product is covered by one or more patents owned by or licensed to Invivoscribe, Inc., including US Patent No. 7,785,783, US Patent No. 8,859,748, US Patent No. 10,280,462 European Patent No. 1549764 (validated in 16 countries, and augmented by related European Patent Nos 2418287 and 2460889), European Patent No. 1633884, Japanese Patent No. 4708029, Japanese Patent No. 6189600, Brazilian Patent No. PI0410283, Canadian Patent No. 2525122, Indian Patent No. 243620, Mexican Patent No. 286493, Chinese Patent No. 1806051, and Korean Patent No. 10-1215194.

These products use nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). Any necessary license to practice amplification methods or to use reagents, amplification enzymes or equipment covered by third party patents is the responsibility of the user and no such license is granted by Invivoscribe, Inc., expressly or by implication.

Research Use Only (RUO) assays are not for sale in Europe and other global markets where equivalent CE-IVD assays are available and registered with the appropriate regulatory agencies.



MRD testing with Next-Generation Sequencing is a proven tool that may be used to develop hematologic malignancy management strategies.

A number of investigators have described NGS-based approaches that have demonstrated success in detecting and monitoring MRD in Chronic Lymphocytic Leukemia (CLL), Acute Lymphoblastic Leukemia (ALL) and other lymphoid malignancies.^{1,2} LymphoTrack Assays are NGS-based deep sequencing assays that detect virtually all clonal rearrangements within targeted T-cell receptor (*TCR*) or immunoglobulin (Ig) antigen receptor loci. Once a specific rearrangement (the clonotype) has been identified, LymphoTrack assays can be used to track these clonotype populations to a sensitivity as low as 10^{-6} . The LymphoTrack MRD solution includes assays, controls and software to facilitate objective MRD assessments over time.

B- or T-cell bundles may be purchased that include the LymphoTrack Assay, LymphoTrack MRD Bioinformatics Software, a LymphoTrack Low Positive Control and a LymphoQuant Internal Control. Each bundle facilitates the standardization of MRD testing by providing controls suitable for longitudinal MRD tracking with test sensitivity assurance. LymphoTrack MRD Software further simplifies clonal tracking due to rich sequence specific data analyses. This software enables objective monitoring of clonal populations by providing multiple functionalities to the user including project planning features and automated bioinformatics applications.

When monitoring MRD, a highly sensitive detection method such as NGS-based LymphoTrack may aid in the early detection of lymphoproliferative disease relapse. However, MRD test results are dependent on DNA amounts interrogated, as well as the confidence level of the test. Controls tracking MRD test sensitivity are thus necessary when reporting MRD test results. Designed for MRD testing, the LymphoTrack Low Positive Control confirms the sensitivity of respective LymphoTrack MRD runs match or exceed a 10^{-4} (or 1 in 10,000) level. Detection of the LymphoTrack Low Positive Control thus lessens false negative reporting concerns at 10^{-4} , and is further necessary to report MRD negative results with confidence at 10^{-4} .

Consistent use of a spike-in internal control enables objective monitoring of clonality over time and test standardization. Invivoscribe now offers both a LymphoQuant B-cell and T-cell Internal Control. Addition of a spike-in LymphoQuant B-cell or T-cell Internal Control to samples allows the software to convert percent clonal reads into estimated clonal cell equivalents.

Reference | 1. *Leukemia* 27:1659-1665, 2013. | 2. *Blood* 120:5173-5180, 2012.



Minimal Residual Disease (MRD) Software

Software Use

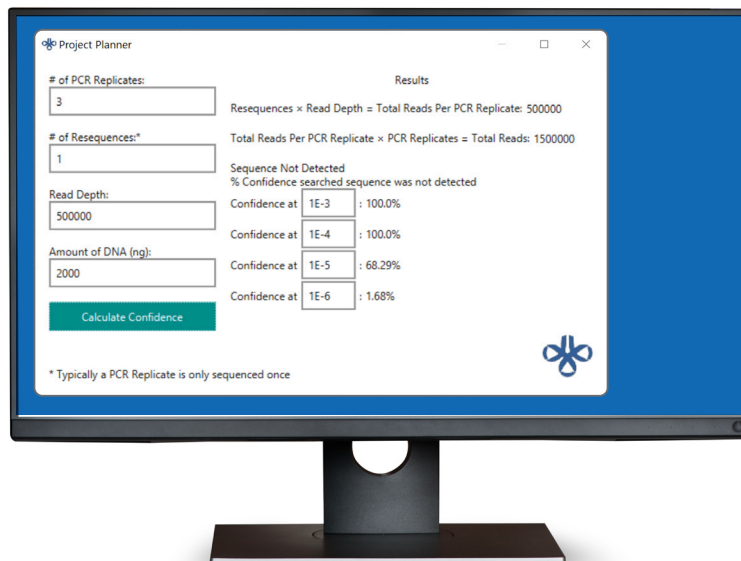
The LymphoTrack MRD Bioinformatics Software package is provided with each LymphoTrack Assay upon request to aid in the evaluation of treatment response in many lymphoid malignancies such as Acute Lymphoblastic Leukemia and Multiple Myeloma to minimize the risk of patients relapsing. The exceptional sensitivity and precision of NGS-based MRD tracking can accelerate clinical trials and drug development. This MRD software is intended to detect the presence of clonotype sequences within the output files generated using the Invivoscribe LymphoTrack Assays and accompanying LymphoTrack bioinformatics software; it is not intended to define the significance of these findings. Once a specific rearrangement sequence (the clonotype) has been identified in a primary sample, the MRD software enables streamlined tracking of clonal populations at a sensitivity of 10^{-4} , or even lower limits provided sufficient DNA is tested. The MRD software can be used to Create, Save and Load projects to objectively track up to 5 clonal sequences to monitor for disease relapse and for use in drug development studies.

The provided software is composed of four distinct parts:

1. A Project tool that can be used to plan experiments with sufficient confidence based on read depth, replicate count, and DNA input. Projects can also be Saved and Loaded for use in subsequent time points.
2. A bioinformatics data analysis application.
3. A PDF Sample Report identifying the clonal sequence(s) if present, a summary of the degree of mismatches, calculations of the read frequency and the degree of confidence if clonal sequence(s) are not detected at various sensitivities.
4. A PDF Summary Report that will automatically generate longitudinal graphs of up to 5 clonal frequencies for a Subject and summary tables with estimates of clonal cell equivalents and clonal frequencies for all queried sequences if LymphoQuant Internal Controls is used.

MRD Project Planner

The Project Planner can be used to calculate the confidence of a true negative by adding replicate counts, resequencing counts, sequencing depth, and DNA input amount. The software assumes that the same sequencing depth and DNA input is used for each replicate.



Research Use Only (RUO) assays are not for sale in Europe and other global markets where equivalent CE-IVD assays are available and registered with the appropriate regulatory agencies.

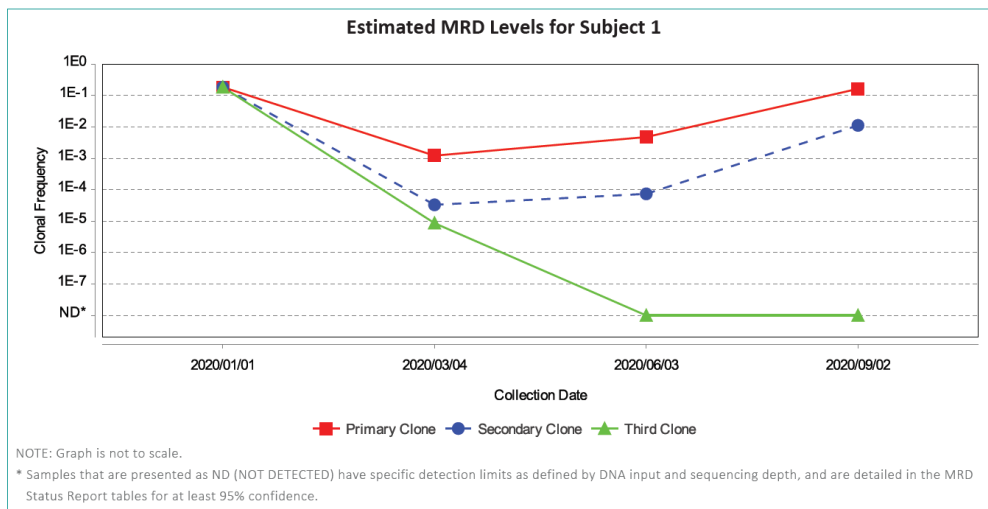
LymphoTrack MRD Sample Report

This report summarizes the overall call, i.e. if a clonotype was detected or not detected, the number of checked replicates, the total DNA input, total reads analyzed and the location of all output files. The values in the PDF report are also found in the generated text files.

Sequence #3 Details for Subject 1 for Collection/Timepoint: 2020/06/03

Sequence Name	PCR Replicate(s)	Total Reads	Gene Target	MRD Result
Third Clone	1	762940	IGH FR1	NOT DETECTED
GCGTCTGGATTTCATTTTCCCTAATGGACAGCCTGAGAGCCGAGGACACGGGTGTGTATAAGTGTGCGAGAAATAGCGTGATGGAAATGCGTGCTTCGTGGTGTCTGGGGCATAGGAGCCAC				
PCR Replicate Details	Cumulative Target Read Count	Cumulative % Total Reads	Cumulative LymphoQuant Read Count	Clonal Frequency
Exact Match	0	0%	100	0
1 Mismatch	1	0.0002%	103	0
2 Mismatch	1	0.0002%	105	0
Detection Limit	% Confidence		Detection Limit	% Confidence
1E-3	99.999%		1E-5	53.761%
1E-4	99.999%		1E-6	2.004%

MRD Summary Graph



Note | The MRD Report may slightly differ from what is shown.

Ordering Information

Catalog #	Products	Quantity Components
7-500-0008	LymphoTrack® MRD Software**	Complimentary with LymphoTrack Assay purchase

** MRD Software can be used to track sequences generated using either LymphoTrack® Assays formatted for either the Illumina® or Thermo Fisher® NGS platforms. MRD applications are for Research Use Only (RUO). Not intended for diagnostic purposes.



Low Positive Controls

Minimal Residual Disease (MRD) testing is a valuable tool that allows investigators to study and monitor multiple myeloma (MM), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML) and other hematologic diseases. Recent treatment advances have led to significantly increased clinical response and overall survival, but ultimately most subjects will relapse, driving the need for sensitive MRD monitoring. Sensitive and standardized testing such as NGS-based MRD may one day enable identification of those cases that will eventually relapse versus those who are potentially cured. In addition to the need for more sensitive tracking, it is clear that standardized methods are needed. Currently, MRD methods are highly subjective and recommendations are often based on consensus expert-shared knowledge and experience, not on a validated, objective method. Once specific rearrangements have been identified, LymphoTrack assays can be used with LymphoQuant and LymphoTrack Low Positive Controls to track these clonotype populations to a sensitivity as low as 10^{-4} .

LymphoTrack® B-cell Low Positive Control

LymphoTrack® B-cell Low Positive Control can be used as a control for:

Gene Rearrangements: *IGH*
Chromosome Translocations: n/c
Mutations: n/c

Catalog #	Description
4-088-0098	LymphoTrack® B-cell Low Positive Control

LymphoTrack® T-cell Low Positive Control

LymphoTrack® T-cell Low Positive Control can be used as a control for:

Gene Rearrangements: *TRB, TRG*
Chromosome Translocations: n/c
Mutations: n/c

Catalog #	Description
4-088-0108	LymphoTrack® T-cell Low Positive Control

Note | Same product listed on page 130 in DNA controls section.
MRD applications are for Research Use Only (RUO). Not intended for diagnostic purposes.

LymphoQuant[®]

Internal Controls

LymphoQuant T-cell or B-cell Internal Controls may be spiked into specimens to estimate the respective number of clonotype T-cell or *IGH* equivalents present. Addition of the LymphoQuant Internal Control to the specimen PCR facilitates clonotype tracking over time without any additional sequencing cost. Consistent use of a LymphoQuant Internal Control enables investigators to objectively monitor the disease over time with a highly standardized, sensitive method. The LymphoTrack MRD software will help researchers that use the LymphoQuant Internal Control, calculate and report an estimated number of clonotype cell equivalents and the percent clonotype in the sample, enabling researchers and pharmaceutical companies to accurately monitor hematologic disease in longitudinal studies.

LymphoQuant[®] B-cell Internal Control

LymphoQuant B-cell Internal Control can be used to objectively track Ig clonotypes.

Gene Rearrangements: *IGH*
Chromosome Translocations: n/c
Mutations: n/c

Catalog #	Description
4-088-0118	LymphoQuant [®] B-cell Internal Control

LymphoQuant[®] T-cell Internal Control

LymphoQuant T-cell Internal Control can be used to objectively track TCR clonotypes.

Gene Rearrangements: *TRB, TRG*
Chromosome Translocations: n/c
Mutations: n/c

Catalog #	Description
4-088-0128	LymphoQuant [®] T-cell Internal Control

Note | Same product listed on page 131 in DNA controls section.
MRD applications are for Research Use Only (RUO). Not intended for diagnostic purposes.



CAPILLARY - CE-IVD ASSAYS

B-Cell Assays

64 IdentiClone® *IGH + IGK* B-Cell Clonality Assays

66 IdentiClone® *IGH* Gene Clonality Assays

68 IdentiClone® *IGK* Gene Clonality Assays

70 IdentiClone® *IGL* B-Cell Clonality Assays

T-Cell Assays

72 IdentiClone® *TCRB* Gene Clonality Assays

74 IdentiClone® T-Cell Receptor Gama Gene Rearrangement Assay 2.0

76 IdentiClone® *TCRD* Gene Clonality Assays

Warranty and Liability

Invivoscribe, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe® shall have no liability for direct, indirect, consequential or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

Notice

This product is covered by one or more patents including European Patent No. 1549764, European Patent No. 2418287, European Patent No. 2460889, Japanese Patent No. 4708029, United States Patent No. 8859748 and United States Patent No. 10280462. All of these patents are licensed to Invivoscribe®.

These products require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). No license under these patents to use amplification processes or enzymes is conveyed expressly or by implication to the purchaser by the purchase of these products.

IdentiClone® is a registered trademark of Invivoscribe®.



IdentiClone Assay kits are CE-marked *in vitro* diagnostic products.*

These kits are intended for PCR-based detection of clonal gene rearrangements and translocations in patients with suspected lymphoproliferations, using gel or capillary electrophoresis methods.

The Invivoscribe CE-marked IdentiClone Assays represent a simple approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was performed at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action.¹ These PCR-based tests include standardized Instructions For Use (IFUs) with interpretation guidelines describing the use of the kits' master mixes and controls. A single thermal cycler program and similar detection methods are used within each IdentiClone kit to improve consistency, reduce human error, and facilitate cross-training.

For more information, please visit www.invivoscribe.com

Reference | 1. JJM van Dongen et al., (2003) *Leukemia* 17:2257-2317.

Disclaimer | IdentiClone Assays are *in vitro* diagnostic products and are not available for sale or use within North America. For more information regarding the research use only reagents, please see the Gel & Capillary Research Use Only Assays section.

IGH + IGK B-Cell Clonality Assays

Assay Description

The IdentiClone *IGH* + *IGK* B-Cell Clonality Assay is an *in vitro* diagnostic product intended for PCR-based detection of clonal immunoglobulin heavy chain and kappa light chain gene rearrangements in patients with suspect lymphoproliferations. Specifically, the *IGH* + *IGK* B-Cell Clonality Assay can be used to:

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies⁴
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Identify tumor-specific markers (*IGH* and *IGK* gene rearrangements) for post-treatment monitoring
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

These kits include six master mixes to test for rearrangements of both *IGH* and *IGK*. The *IGH* Tube A, B, and C master mixes target the framework 1, 2, 3 regions (respectively) within the variable (V_H) region, and the joining (J_H) region of the immunoglobulin heavy chain locus. The *IGK* Tube A master mix targets the variable (V_k) and the joining (J_k) region. *IGK* Tube B master mix targets kappa deleting element (K_{del}) rearrangements with the variable (V_k) region and the intragenic J_k - C_k region. The resulting V_k - K_{del} and J_k - C_k intron- K_{del} rearrangements are a result of unsuccessful rearrangements retained by the B cell. For best sensitivity, it is recommended to test suspect B-cell malignancies for both *IGH* and *IGK*.³ The included Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

Performance Characteristics

Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision.² The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

PCR/SB concordance:¹

IGH: 93% sensitivity / 92% specificity

IGK: 90% sensitivity / 90% specificity

PCR vs. SB analysis relative to histopathology and final diagnosis:

PCR/SB concordance:² PCR sensitivity: SB sensitivity:

IGH + *IGK*: 85% 98% 39%

References

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).
2. Y Sandberg et al., *J. Mol. Diag.* 7(4):495-503 (2005).
3. Van Krieken, JHJM et al., *Leukemia* 21:201 - 206 (2007).

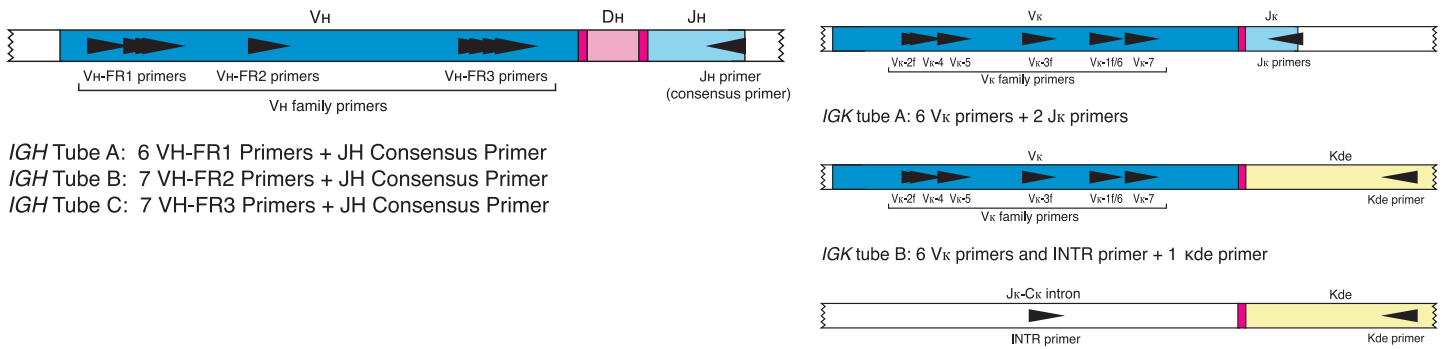


Figure Legend: Simple representation of the organization of a rearranged immunoglobulin heavy chain (*IGH*) gene on chromosome 14q32.33 and the immunoglobulin kappa light chain gene on chromosome 2p11.2. Black arrows represent the relative positions of primers that target the conserved framework regions (FR1-3) and the downstream consensus J_H gene segments for *IGH* and the V_K, J_K, INTR and Kde primers which are included in the *IGK* master mix tubes.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0019 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0007 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
<i>IGH</i> Tube A	Framework 1 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
<i>IGH</i> Tube B	Framework 2 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
<i>IGH</i> Tube C	Framework 3 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
<i>IGK</i> Tube A	V _K -J _K	1 x 1500 µL tube	10 x 1500 µL tubes
<i>IGK</i> Tube B	V _K -K _{de} , Intron-K _{de}	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
9-100-0031	IdentiClone® <i>IGH</i> + <i>IGK</i> B-Cell Clonality Assay - ABI Fluorescence Detection	33 reactions
9-100-0041	IdentiClone® <i>IGH</i> + <i>IGK</i> B-Cell Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.

IGH Gene Clonality Assays

Assay Description

The IdentiClone IGH Gene Clonality Assay is an *in vitro* diagnostic product intended for PCR-based detection of clonal immunoglobulin heavy chain gene rearrangements in patients with suspect lymphoproliferations.

Specifically, the IGH Gene Clonality Assay can be used to:

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies⁴
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Identify tumor-specific markers (IGH gene rearrangements) for post-treatment monitoring
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

These test kits include six master mixes. The IGH Tube A, B, and C master mixes target the framework 1, 2, and 3 regions (respectively) within the variable (V_H) region and the joining (J_H) region of the immunoglobulin heavy chain locus. The IGH Tube D and E master mixes target the diversity and joining regions. The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

Performance Characteristics

Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision.² The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

PCR/SB concordance:¹

IGH: 93% sensitivity / 92% specificity

PCR vs. SB analysis relative to histopathology and final diagnosis:

PCR/SB concordance:²

IGH + IGK: 85%

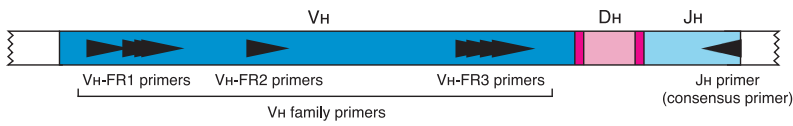
PCR sensitivity: SB sensitivity:

98%

39%

References

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).
2. Y Sandberg et al., *J. Mol. Diag.* 7(4):495-503 (2005).



Tube A: 6 V_H -FR1 Primers + J_H Consensus Primer
 Tube B: 7 V_H -FR2 Primers + J_H Consensus Primer
 Tube C: 7 V_H -FR3 Primers + J_H Consensus Primer



Tube D: 6 D_H Primers + J_H Consensus Primer
 Tube E: D_H 7 Primer + J_H Consensus Primer

Figure Legend: Simple representation of the organization of a rearranged immunoglobulin heavy chain gene on chromosome 14q32.33. Black arrows represent the relative positions of primers that target the conserved framework (FR1-3) and diversity (DH1-7) regions, and the downstream consensus J_H gene segments. The amplicon products generated from each of these regions can be differentially detected when fluorescent primer sets are used with capillary electrophoresis instruments that employ differential fluorescence detection.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0019 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0024 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0008 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
IGH Tube A	Framework 1 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube B	Framework 2 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube C	Framework 3 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube D	D _H 1-6 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube E	D _H 7 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
9-101-0061	IdentiClone® IGH Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
9-101-0081	IdentiClone® IGH Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.

IGK Gene Clonality Assays

Assay Description

The IdentiClone IGK Gene Clonality Assay is an *in vitro* diagnostic product intended for PCR-based detection of clonal immunoglobulin kappa light chain gene rearrangements in patients with suspect lymphoproliferations.

Specifically, the IGK Gene Clonality Assay can be used to:

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Identify tumor-specific markers (IGK and IGK-K_{de} rearrangements) for post-treatment monitoring
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

These test kits include three master mixes. The IGK Tube A master mix targets the variable (V_k) and the joining (J_k) regions of the immunoglobulin kappa light chain locus, whereas the IGK Tube B master mix targets kappa deleting element (K_{de}) rearrangements with the V_k regions and the intragenic J_k-C_k regions. The V_k-K_{de} and J_k-C_k intron-K_{de} rearrangements are a result of unsuccessful rearrangements retained by the B cell. The third master mix, the Specimen Control Size Ladder, targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

Performance Characteristics

Data from two independent studies that tested more than 300 patient sample of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision.² The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

PCR/SB concordance:¹

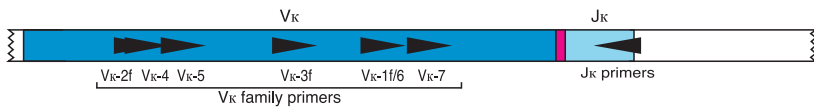
IGK: 90% sensitivity / 90% specificity

PCR vs. SB analysis relative to histopathology and final diagnosis:

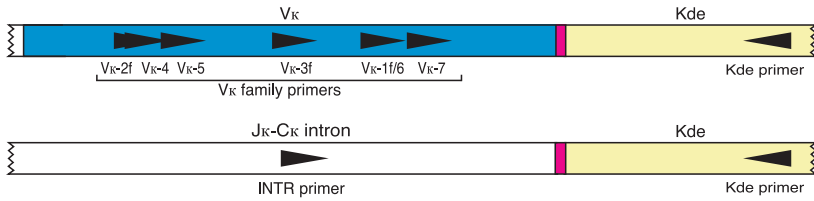
PCR/SB concordance: ²	PCR sensitivity:	SB sensitivity:
IGH + IGK: 85%	98%	39%

References

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).
2. Y Sandberg et al., *J. Mol. Diag.* 7(4):495-503 (2005).



IGK tube A: 6 V κ primers + 2 J κ primers



IGK tube B: 6 V κ primers and INTR primer + 1 Kde primer

Figure Legend: Schematic diagram of the immunoglobulin kappa light chain gene complex on chromosome 2p11.2. Shown are the relative positions and orientations for the V κ -J κ , and K_{de} primers, which are included in the IGK master mix tubes.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0007 Clonal Control DNA	200 $\mu\text{g}/\text{mL}$	1 x 100 μL tube	5 x 100 μL tubes
IVS-0000 Polyclonal Control DNA	200 $\mu\text{g}/\text{mL}$	1 x 100 μL tube	5 x 100 μL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
IGK Tube A	V κ - J κ	1 x 1500 μL tube	10 x 1500 μL tubes
IGK Tube B	V κ -K _{de} , Intron-K _{de}	1 x 1500 μL tube	10 x 1500 μL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 μL tube	10 x 1500 μL tubes

Ordering Information

Catalog #	Products	Quantity
9-102-0021	IdentiClone® IGK Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
9-102-0031	IdentiClone® IGK Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.

IGL Gene Clonality Assays

Assay Description

The IdentiClone *IGL* Gene Clonality Assay is an *in vitro* diagnostic product intended for PCR-based detection of clonal immunoglobulin lambda light chain gene rearrangements in patients with suspect lymphoproliferations and can be used to:

Specifically, the IdentiClone *IGL* Gene Clonality Assays can be used to:

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Identify tumor-specific markers (*IGL* gene rearrangements) for post-treatment monitoring
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

These test kits include two master mixes. The *IGL* Tube master mix targets the variable (V λ) region and the joining (J λ) region of the immunoglobulin lambda light chain gene locus (*IGL*). The Specimen Control Size Ladder targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

Performance Characteristics

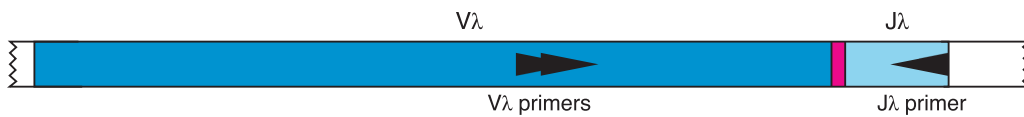
Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision.²The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

PCR/SB concordance:¹

IGL: 86% sensitivity / 92% specificity

References

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).
2. Y Sandberg et al., *J. Mol. Diag.* 7(4):495-503 (2005).



***IGL* tube: 2 $V\lambda$ primers + 1 $J\lambda$ primer**

Figure Legend: Schematic diagram of the immunoglobulin lambda light chain gene complex on chromosome 22q11.2. Shown are the relative positions and orientations for the $V\lambda$ and $J\lambda$ primers, which are included in the *IGL* master mix tube. The two $V\lambda$ primers only target $V\lambda 1$, 2, and 3 because these three V families cover approximately 70% of rearrangeable $V\lambda$ gene segments, and approximately 90% of all *IGL* gene rearrangements involve these three families. Similarly, the single $J\lambda$ primer only targets $J\lambda 1$, 2, and 3 because these three J segments are involved in 98% of all *IGL* gene rearrangements.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0010 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0029 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
<i>IGL</i> Tube	$V\lambda - J\lambda$	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
9-103-0011	IdentiClone® <i>IGL</i> Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
9-103-0021	IdentiClone® <i>IGL</i> Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.

TCRB Gene Clonality Assays

Assay Description

The IdentiClone *TCRB* Gene Clonality Assay is an *in vitro* diagnostic product intended for PCR-based detection of clonal T-cell receptor beta chain gene rearrangements in patients with suspect lymphoproliferations.

Specifically, the *TCRB* Gene Clonality Assay can be used to:

- Identify clonality in suspect lymphoproliferations
- Support a differential diagnosis between reactive lesions and T-cell and some immature B-cell malignancies
- Determine lineage involvement in mature lymphoproliferative disorders
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

These kits include four master mixes. *TCRB* Tubes A and B target framework regions within the variable region, and the joining region ($V\beta$) of the *TCR* beta chain locus. *TCRB* Tube C targets the diversity and joining ($J\beta$) regions of the *TCR* beta chain locus. The Specimen Control Size Ladder master mix included targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

Performance Characteristics

Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision.² The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

PCR/SB concordance:¹

TRB: 86% sensitivity / 98% specificity

PCR vs. SB analysis relative to histopathology and final diagnosis:

	PCR/SB concordance: ²	PCR sensitivity:	SB sensitivity:
TRB:	85%	96%	35%

References

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).
2. Y Sandberg et al., *J. Mol. Diag.* 7(4):495-503 (2005).



Tube A: 23 Vβ primers + 6 Jβ1 primers and 3 Jβ2 primers

Tube B: 23 Vβ primers + 4 Jβ2 primers

Tube C: 2 Dβ primers + 13 Jβ primers



Figure Legend: Simplified diagram of a representative rearranged T-cell receptor beta gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for Master Mix Tubes A, B, and C.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0009 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0004 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0021 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
TCRB Tube A	Multiple Vβ + Jβ1/2	1 x 1500 µL tube	10 x 1500 µL tubes
TCRB Tube B	Multiple Vβ + Jβ2	1 x 1500 µL tube	10 x 1500 µL tubes
TCRB Tube C	Multiple Dβ + Jβ1/2	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
9-205-0011	IdentiClone® TCRB Clonality Assay - ABI Fluorescence Detection	33 reactions
9-205-0021	IdentiClone® TCRB Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.

T-Cell Receptor Gamma Gene Rearrangement Assay 2.0

Assay Description

The IdentiClone T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 is an *in vitro* diagnostic product intended for PCR-based detection of clonal T-cell receptor gamma chain gene rearrangements in patients with suspect lymphoproliferations.

Specifically, the T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 can be used to identify clonality in suspect lymphoproliferations.

Summary and Explanation of the Test

Rearrangements of the antigen receptor genes occur during ontogeny in B- and T-lymphocytes. These gene rearrangements generate products that are unique in length and sequence. Polymerase chain reaction (PCR) assays can be used to identify lymphocyte populations derived from a single cell by detecting the unique V-J gene rearrangements present within these antigen receptor loci.¹

This assay allows for amplification of the *TRG* region with fluorescent labeled primers, yielding products that can be grouped under a single Gaussian distribution when separated by size using capillary electrophoresis. In addition, the product size facilitates increased success when testing FFPE samples. The included analysis algorithm aids in the interpretation of data and identification of significant clonal peaks. Presence or absence of molecular clonality can support the differential diagnosis of reactive lesions and certain B- and T-cell malignancies, provided that the results are interpreted in the context of all available clinical, histological, and immunophenotypic data.

Performance Characteristics

To assess the performance of the *TCRG* 2.0 Assay, testing was performed on cell lines with known clonal rearrangements followed by testing on previously sequenced clinical samples.

When used in combination with the provided *TCRG* Algorithm worksheet, the assay was capable of detecting DNA from 6 control cell lines (200 ng/ μ L) diluted into polyclonal tonsil DNA (200 ng/ μ L) at 5% (v/v).

Furthermore, the performance of the *TCRG* 2.0 Assay was evaluated on clinical samples for which the T-cell receptor gamma gene rearrangement status had been identified by Roche 454 sequencing. For the 7 samples that had been identified as clonal by sequencing, the *TCRG* 2.0 assay had 100% concordance. For the 12 samples that were either negative for a clonal event or were oligoclonal, concordance of the *TCRG* 2.0 assay was 75%. Sample types included peripheral blood, bone marrow, and formalin-fixed, paraffin embedded (FFPE) tissue.

Always interpret the results of molecular clonality tests in the context of clinical, histological and immunophenotypic data.

References

1. Miller JE, Wilson SS, Jaye DJ, and Kronenberg M. *Mol. Diag.* 1999, 4(2):101-117.
2. Armand, Marine et al. *HemaSphere*, 2019;3:3.

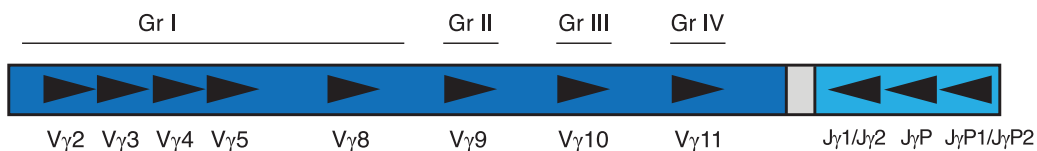


Figure Legend: Simple representation of the organization of the T-cell receptor gamma gene on chromosome 7p14. Black arrows represent the relative positions of primers that target the variable region genes and the downstream joining region gene segments that are involved in rearrangements in T-cell lymphomas. The downstream primers are fluorescently labeled through the incorporation of a 6FAM fluorophore. The amplicon products generated from these rearrangements are detected by capillary electrophoresis.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
5% TCRG Positive Control DNA	50 µg/mL	1 x 50 µL tube	5 x 50 µL tube
TCRG Negative Control DNA	50 µg/mL	1 x 50 µL tube	5 x 50 µL tube
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
TCRG - 6FAM	Vγ1-Vγ11 + Jγ1/Jγ2, JγP, JγP1/JγP2	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
9-207-0101	IdentiClone® T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 - ABI Fluorescence Detection	33 reactions
9-207-0111	IdentiClone® T-Cell Receptor Gamma Gene Rearrangement Assay MegaKit 2.0 - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.



TCRD Gene Clonality Assays

Assay Description

The IdentiClone *TCRD* Gene Clonality Assay is an *in vitro* diagnostic product intended for PCR-based detection of clonal T-cell receptor delta chain gene rearrangements in patients with suspect lymphoproliferations and can be used to:

- Identify clonality in suspect lymphoproliferations
- Support a differential diagnosis between reactive lesions and T-cell and some immature B-cell malignancies
- Determine lineage involvement in mature lymphoproliferative disorders
- Monitor and evaluate disease recurrence

Summary and Explanation of the Test

These test kits include two master mixes. The *TCRD* tube targets the framework regions within the variable region, the diversity region, and the joining region of the T-cell receptor delta chain locus (*TRD*, formerly known as *TCRD*). The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

Performance Characteristics

Data from an independent, peer-reviewed study suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. There were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision. The clinico- histopathological diagnosis correlates well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

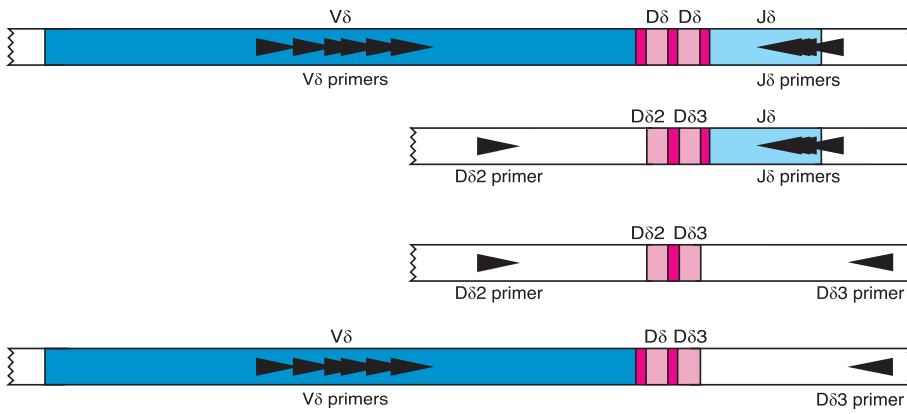
PCR/SB concordance:¹
TRD: 83% sensitivity / 95% specificity

Reference

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).



This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.



TCRD tube: 6 V δ and 1 D δ 2 primers + 4 J δ and 1 D δ 3 primers

Figure Legend: Simplified diagram of a representative rearranged T-cell receptor delta gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for the TRD Tube Master Mix tube.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0021 Clonal Control DNA	200 μ g/mL	1 x 100 μ L tube	5 x 100 μ L tubes
IVS-0000 Polyclonal Control DNA	200 μ g/mL	1 x 100 μ L tube	5 x 100 μ L tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
TCRD Tube	Multiple V δ + D δ + J δ	1 x 1500 μ L tube	10 x 1500 μ L tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 μ L tube	10 x 1500 μ L tubes

Ordering Information

Catalog #	Products	Quantity
9-206-0011	IdentiClone® TCRD Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
9-206-0021	IdentiClone® TCRD Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.

LeukoStrat[®]

CAPILLARY DETECTION - CE-IVD ASSAYS AND IVD COMPANION DIAGNOSTIC ASSAYS

FLT3 Assays

80 FLT3 Mutation Assay 2.0 - ABI Fluorescence Detection

Companion Diagnostic Assays

82 LeukoStrat CDx FLT3 Mutation Assay (IVD) - United States

84 LeukoStrat CDx FLT3 Mutation Assay (CE-IVD)

86 LeukoStrat CDx FLT3 Mutation Assay - Japan

Warranty and Liability

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Use of this product may require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). Any necessary license to practice amplification methods or to use reagents, amplification enzymes or equipment covered by third party patents is the responsibility of the user and no such license is granted by Invivoscribe, Inc., expressly or by implication.

Disclaimer

The products in the section that follows are *in vitro* diagnostic products. CE-IVD products are not available for sale or use within North America.

LeukoStrat[®]

LeukoStrat Assay Kits are *in vitro* diagnostic products intended for PCR-based detection of *FLT3* activating mutations (ITD, TKD) in patients with acute myelogenous leukemia (AML) using capillary electrophoresis methods, and include companion diagnostic assays.

All tests include PCR master mixes and controls for ITD and TKD detection, along with Instructions For Use (IFUs). Master mixes are composed of a buffered magnesium chloride solution, deoxynucleotides, and primers targeting the gene segments of interest. These assay master mixes are complete other than Taq DNA polymerase.

Companion Diagnostics

The LeukoStrat CDx *FLT3* Mutation Assays are companion diagnostics, and provide a complete solution with software automated PDF reports including mutant identification, mutant to wild type signal ratios, and clinical interpretation. Additional reagents of Taq DNA Polymerase (US, JP), EcoRV Enzyme (US, JP), and NEBuffer r3.1 (US, JP) are included in IVD kits.

Disclaimer | LeukoStrat Assays are *in vitro* diagnostic products. CE-IVD products are not available for sale or use within North America. IVD products are available for sale or use where registered. Refer to product page for respective detail. For more information regarding the research use only reagents, please see the Gel & Capillary Research Use Only Assays section.

LeukoStrat[®]

FLT3 Mutation Assay 2.0

ABI Fluorescence Detection

Assay Description

The LeukoStrat[®] FLT3 Mutation Assay 2.0 is an *in vitro* diagnostic product intended for PCR-based detection of FLT3 activating mutations in patients with acute myelogenous leukemia (AML). Specifically, the FLT3 Mutation Assay 2.0 can be used to:

1. Identify internal tandem duplications (ITD) in the FLT3 gene
2. Identify tyrosine kinase domain (TKD) mutations in the FLT3 gene

Summary and Explanation of the Test

AML in general has a poor prognosis.^{1,2} Many studies in AML have shown that the presence of FLT3 (fms related tyrosine kinase 3) activating mutations portends a poor prognosis making it an attractive target for treatment. For this reason, FLT3 mutation testing is required to stratify disease and determine appropriate treatment options.

Using this assay, DNA is amplified via PCR with fluorophore labeled primers, TKD amplicon is enzymatically digested, and FLT3 mutations are detected via capillary electrophoresis. This test kit includes 2 PCR master mixes, along with positive and negative controls for mutant detection. Each master mix (FLT3 ITD Master Mix and FLT3 D835 Master Mix) contains a fluorophore-labeled PCR primer set for the respective detection of internal tandem duplication or TKD mutation.

Performance Characteristics

This assay can reliably detect mutations comprising more than 5% of the total cell population. Also, as demonstrated herein, the LeukoStrat FLT3 Mutation Assay 2.0 detects FLT3 ITD and TKD mutations with excellent concordance to NGS methodologies (Table 1, Table 2).

Table 1. FLT3 ITD Percent Agreement with 454 Sequencing

Percentage Agreement	Discordance #	Concordance #	*95% LL
Negative PA 100%	0	119	96.9%
Postive PA 98.0%	4	200	95.1%

*95% of results would be expected to agree with sequencing at a rate greater than or equal to the lower limit (LL).

Table 2. FLT3 TKD Percent Agreement with 454 Sequencing

Percentage Agreement	Discordance #	Concordance #	*95% LL
Negative PA 100%	0	137	96.9%
Postive PA 100%	0	240	98.5%

*95% of results would be expected to agree with sequencing at a rate greater than or equal to the lower limit (LL).

References

1. Murphy KM et al., A Clinical PCR/Capillary Electrophoresis Assay for the Detection of Internal Tandem Duplication and Point Mutation of the FLT3 Gene. *J. Mol. Diag.* 5:96-102 (2003).
2. Yamamoto, Y., et al., Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. *Blood*, 97(8):2434-9 (2001).
3. Acute Myeloid Leukemia, Clinical Practice Guidelines in Oncology, National Comprehensive Cancer Network (v.2.2014)

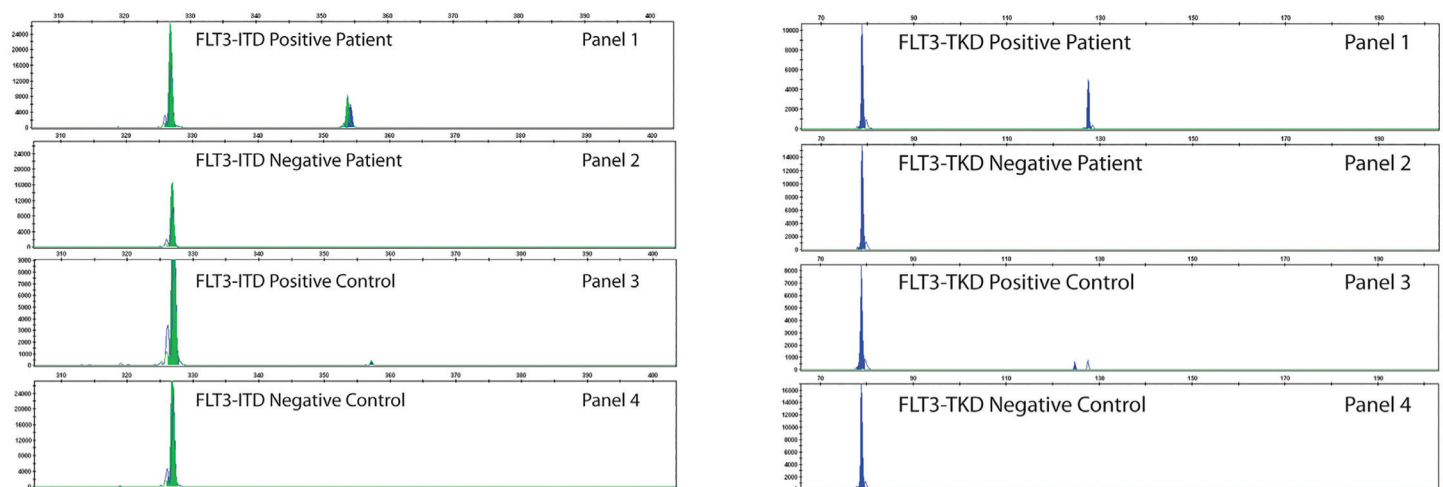
Reagents

Controls	Concentration	Units in Assay	Units in MegaKit
<i>FLT3</i> ITD Positive Control	50 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
<i>FLT3</i> D835 Positive Control	50 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
<i>FLT3</i> Negative Control	50 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in MegaKit
<i>FLT3</i> ITD Master Mix – 6FAM & HEX	<i>FLT3</i> ITD	1 x 1500 µL tube	10 x 1500 µL tubes
<i>FLT3</i> D835 Master Mix – 6FAM	<i>FLT3</i> TKD	1 x 1500 µL tube	10 x 1500 µL tubes

Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI fluorescence detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in high sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.

The data shown was generated using the master mixes indicated. Amplified products were run on an ABI 3500xL instrument.



Ordering Information

Catalog #	Products	Quantity
9-412-0091	LeukoStrat® <i>FLT3</i> Mutation Assay 2.0 – ABI Fluorescence Detection	33 reactions
9-412-0101	LeukoStrat® <i>FLT3</i> Mutation Assay 2.0 MegaKit – ABI Fluorescence Detection	330 reactions

These are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.

LeukoStrat[®]

CDx *FLT3* Mutation Assay

FDA Approved Companion Diagnostic

Available in the USA - *In Vitro* Diagnostic Kit PN K4120361

FDA approved assay for assessment of acute myeloid leukemia (AML) patients eligible for treatment with RYDAPT[®] (midostaurin) or XOSPATA[®] (gilteritinib fumarate), now available as US distributed kit. This *FLT3* companion diagnostic includes reagents along with software that identifies ITD and TKD mutations, generates mutant/wildtype signal ratios, and predicts response to gilteritinib and midostaurin.

Clinical Significance of *FLT3* Mutation Status: Each year approximately 21,000 patients in the United States are diagnosed with AML. Of those diagnosed with AML, ~1 out of 3 are expected to have presence of *FLT3* mutations, (*FLT3*mut+). Since *FLT3*mut+ AML is clinically actionable, stratification of AML patients by testing for *FLT3* mutation status has become a standard of care.

Intended Use

The LeukoStrat CDx *FLT3* Mutation Assay is a PCR-based *in vitro* diagnostic test designed to detect internal tandem duplication (ITD) and tyrosine kinase domain (TKD) mutations D835 and I836 in the *FLT3* gene in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myelogenous leukemia (AML).

The LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom RYDAPT[®] (midostaurin) treatment is being considered.

The LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom XOSPATA[®] (gilteritinib) treatment is being considered.

The test is for use on the 3500xL Dx Genetic Analyzer.

Summary and Explanation of the Test

Acute myelogenous leukemia (AML) in general has a poor prognosis. Many studies in AML have shown that the presence of *FLT3* activating mutations portends a poor prognosis making it an attractive target for treatment.^{1,2}

The LeukoStrat CDx *FLT3* Mutation Assay targets regions of the *FLT3* gene to identify internal tandem duplication (ITD) mutations and tyrosine kinase domain (TKD) mutations, such as the D835 and I836 mutations.

The LeukoStrat CDx *FLT3* Mutation Assay includes reagents, software and procedures for isolating mononuclear cells and extracting DNA from patient peripheral blood or bone marrow specimens to determine if *FLT3* mutations are present.

DNA is amplified via PCR and the amplicons are detected via capillary electrophoresis. *FLT3* mutation status is determined by the LeukoStrat CDx *FLT3* Software. A *FLT3* ITD and/or TKD mutation is reported as Positive if the mutant:wild-type signal ratio meets or exceeds the clinical cutoff of 0.05.

Method Description

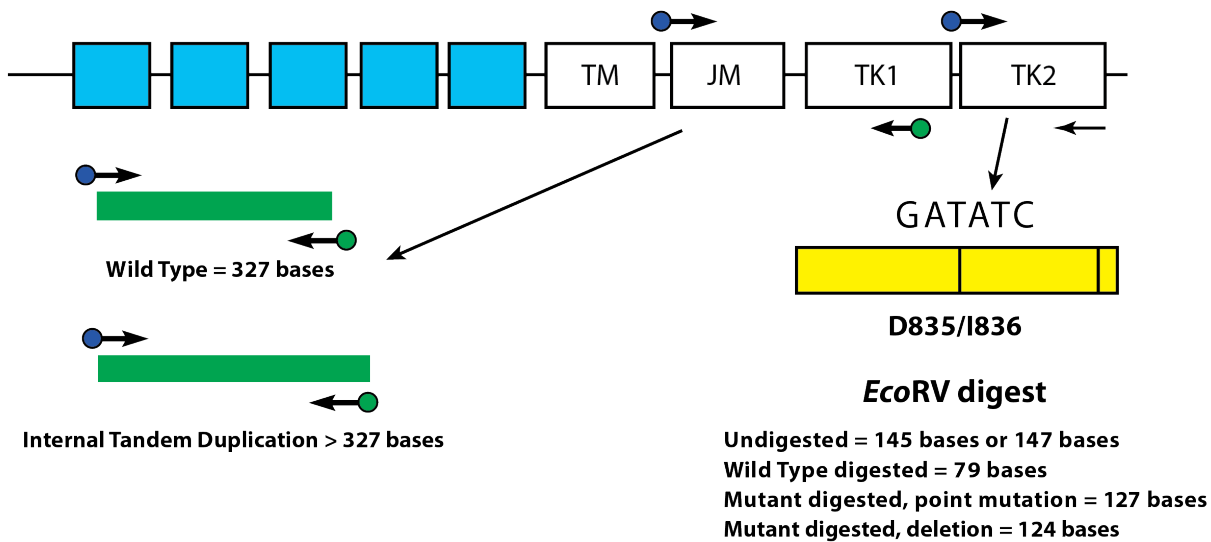
ITD Mutations of *FLT3*

The LeukoStrat CDx *FLT3* Mutation Assay uses fluorescently labeled primers that are in and around the JM region. Wild-type *FLT3* alleles will amplify and produce a product at 327±1 bp as measured by this assay, while alleles that contain ITD mutations will produce a product that exceeds 327±1 bp (see Figure, right).

TKD Mutations of *FLT3*

The LeukoStrat CDx *FLT3* Mutation Assay uses primers that lie on either side of the TKD region. The *FLT3* target region is amplified using PCR and then an EcoRV restriction digest is performed. Wild-type alleles of the *FLT3* gene yield digestion products of 79±1 bp whereas mutant alleles yield products of 125±1 bp or 127±1 bp from the original undigested amplicon product of 145±1 bp or 147±1 bp, as measured by this assay (see Figure, right).

Method Schematic: *FLT3* ITD & TKD Mutant Detection



Depicted is a representation of the *FLT3* juxtamembrane (JM) region (TM = transmembrane) and the activating loop of the tyrosine kinase (TK) domain. Black arrows represent the relative positions of primers that target in and around the JM region for ITD or the activating loop of the kinase domain for TKD. Colored dots represent fluorophores on labeled primers. The yellow box has vertical black lines that represent the position of the EcoRV restriction digest sites.

Reagents

Reagent Name	Units in Assay
<i>FLT3</i> Extraction Control	1 x 1800 µL tube
<i>FLT3</i> ITD Master Mix	1 x 1500 µL tube
<i>FLT3</i> TKD Master Mix	1 x 1500 µL tube
<i>FLT3</i> ITD Positive Control	1 x 100 µL tube
<i>FLT3</i> TKD Positive Control	1 x 100 µL tube
<i>FLT3</i> No Template Control	1 x 200 µL tube
Taq DNA Polymerase	1 x 200 µL tube
EcoRV Enzyme	1 x 200 µL tube
NEBuffer r3.1	1 x 1250 µL tube



Ordering Information

Catalog #	Products	Quantity
K-412-0361	LeukoStrat® CDx <i>FLT3</i> Mutation Assay	33 reactions
K-412-0371	LeukoStrat® CDx <i>FLT3</i> Software	CD complimentary with purchase
K-412-0401	LeukoStrat® CDx Assay Installer	USB complimentary with purchase

References

- Murphy KM et al., A Clinical PCR/Capillary Electrophoresis Assay for the Detection of Internal Tandem Duplication and Point Mutation of the *FLT3* Gene. *J. Mol. Diag.* 5:96-102 (2003).
- Yamamoto, Y., et al., Activating mutation of D835 within the activation loop of *FLT3* in human hematologic malignancies. *Blood*, 97(8):2434-9 (2001).

These are *in vitro* diagnostic (IVD) products, and are available for sale or use in the United States only

LeukoStrat[®]

CDx *FLT3* Mutation Assay

CE-marked Companion Diagnostic

The only internationally standardized CE-IVD assay for *FLT3* Signal Ratio mutation analysis for assessment of acute myeloid leukemia (AML) patients eligible for treatment with RYDAPT[®] (midostaurin) or XOSPATA[®] (gilteritinib fumarate).

Intended Use

The LeukoStrat CDx *FLT3* Mutation Assay is a PCR-based *in vitro* diagnostic test designed to detect internal tandem duplications (ITD) and tyrosine kinase domain (TKD) mutations D835 and I836 in the *FLT3* gene in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myelogenous leukemia (AML).

In regions where midostaurin is available, the LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom RYDAPT[®] (midostaurin) treatment is being considered.

In regions where gilteritinib fumarate is available, the LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom XOSPATA[®] (gilteritinib fumarate) treatment is being considered.

Summary and Explanation of the Test

AML in general has a poor prognosis. Assessment of the mutation status of the *FLT3* (fms related tyrosine kinase 3) receptor gene in karyotype normal AML is the most important prognostic indicator of disease outcome, which is often substantial, as many studies in AML have shown that the presence of *FLT3* activating mutations portends a poor prognosis.^{1,2}

The LeukoStrat CDx *FLT3* Mutation Assay targets regions of the *FLT3* gene to identify ITD mutations and TKD mutations, such as the D835 and I836 mutations, and has been validated in an international clinical trial.

The LeukoStrat CDx *FLT3* Mutation Assay includes reagents, equipment, software and procedures for isolating mononuclear cells and extracting DNA from patient specimens to determine if *FLT3* mutations are present. DNA is amplified via PCR and the amplicons are detected via capillary electrophoresis. *FLT3* mutation status is determined by the LeukoStrat CDx *FLT3* Software. A *FLT3* ITD and/or TKD mutation is reported as Positive if the mutant:wild-type signal ratio meets or exceeds the clinical cutoff of 0.05.

Method Description

ITD Mutations of *FLT3*

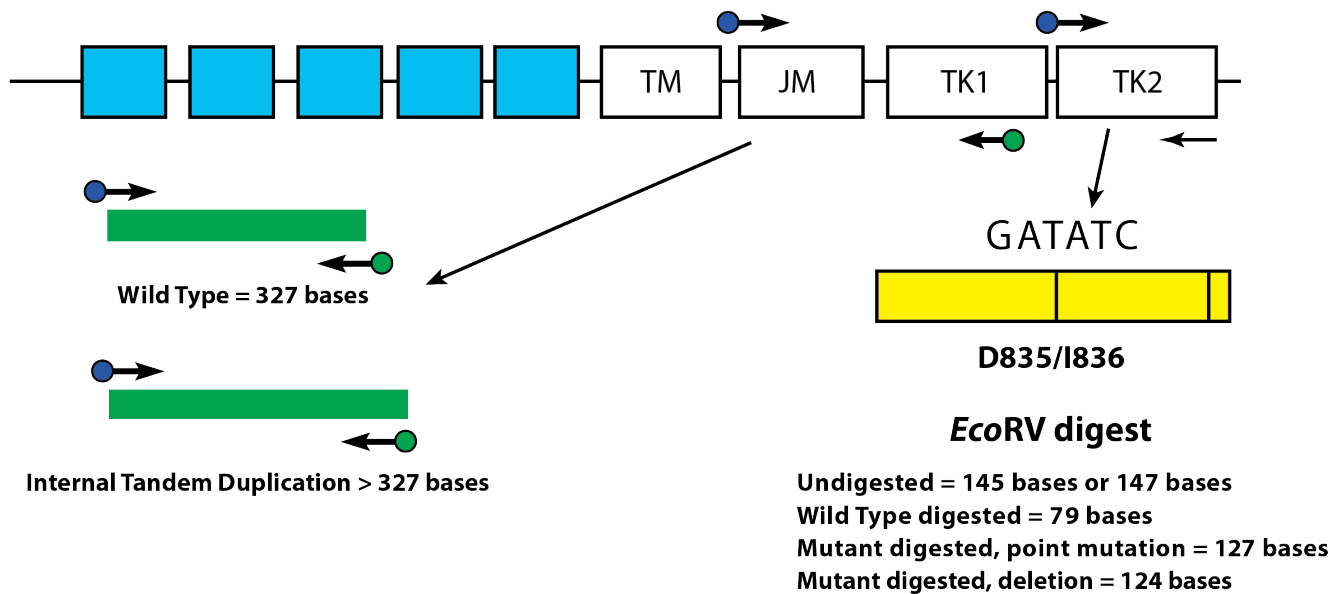
The LeukoStrat CDx *FLT3* Mutation Assay uses fluorescently labeled primers that are in and around the JM region. Wild-type *FLT3* alleles will amplify and produce a product at 327±1 bp as measured by this assay, while alleles that contain ITD mutations will produce a product that exceeds 327±1 bp (see Figure, right).

TKD Mutations of *FLT3*

The LeukoStrat CDx *FLT3* Mutation Assay uses primers that lie on either side of the TKD region. The *FLT3* target region is amplified using PCR and then an EcoRV restriction digest is performed. Wild-type alleles of the *FLT3* gene yield digestion products of 79±1 bp whereas mutant alleles yield products of 125±1 bp or 127±1 bp from the original undigested amplicon product of 145±1 bp or 147±1 bp, as measured by this assay (please see Figure, right).

References

1. Murphy KM et al., A Clinical PCR/Capillary Electrophoresis Assay for the Detection of Internal Tandem Duplication and Point Mutation of the *FLT3* Gene. *J. Mol. Diag.* 5:96-102 (2003).
2. Yamamoto, Y., et al., Activating mutation of D835 within the activation loop of *FLT3* in human hematologic malignancies. *Blood*, 97(8):2434-9 (2001).



Depicted is a representation of the *FLT3* juxtamembrane (JM) region (TM = transmembrane) and the activating loop of the tyrosine kinase (TK) domain. Black arrows represent the relative positions of primers that target in and around the JM region for ITD or the activating loop of the kinase domain for TKD. Colored dots represent fluorophores on labeled primers. The yellow box has vertical black lines that represent the position of the *EcoRV* restriction digest sites.

Reagents

Controls	Units in Assay
<i>FLT3</i> Extraction Control	1 x 1800 µL tube
<i>FLT3</i> ITD Master Mix	1 x 1500 µL tube
<i>FLT3</i> TKD Master Mix	1 x 1500 µL tube
<i>FLT3</i> ITD Positive Control	1 x 100 µL tube
<i>FLT3</i> TKD Positive Control	1 x 100 µL tube
<i>FLT3</i> No Template Control	1 x 200 µL tube

Ordering Information

Catalog #	Products	Quantity
K-412-0291	LeukoStrat® CDx <i>FLT3</i> Mutation Assay	33 reactions
K-412-0281	LeukoStrat® CDx <i>FLT3</i> Software	1 CD complimentary with purchase

These are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.

Available in Japan

The only internationally standardized assay for *FLT3* Signal Ratio mutation analysis for assessment of acute myeloid leukemia (AML) patients eligible for treatment with Gilteritinib Fumarate or Quizartinib Hydrochloride.

Intended Use

The LeukoStrat CDx *FLT3* Mutation Assay is a PCR-based, *in vitro* diagnostic test designed to detect internal tandem duplication (ITD) mutations and tyrosine kinase domain (TKD) mutations D835 and I836 in the *FLT3* gene in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myelogenous leukemia.

The LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom Gilteritinib Fumarate treatment is being considered.

The LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom Quizartinib Hydrochloride treatment is being considered.

Summary and Explanation of the Test

AML in general has a poor prognosis. Assessment of the mutation status of the *FLT3* (fms related tyrosine kinase 3) receptor gene in karyotype normal AML is the most important prognostic indicator of disease outcome, which is often substantial, as many studies in AML have shown that the presence of *FLT3* activating mutations portends a poor prognosis.^{1,2} The LeukoStrat CDx *FLT3* Mutation Assay targets regions of the *FLT3* gene to identify ITD mutations and TKD mutations, such as the D835 and I836 mutations, and has been validated in an international clinical trial.

The LeukoStrat CDx *FLT3* Mutation Assay includes reagents, equipment, software and procedures for isolating mononuclear cells and extracting DNA from patient specimens to determine if *FLT3* mutations are present. DNA is amplified via PCR with fluorescently labeled primers, amplicon is enzymatically digested (TKD), and the amplicons are detected via capillary electrophoresis. *FLT3* mutation status is determined by the LeukoStrat CDx *FLT3* Software. A *FLT3* ITD and/or TKD mutation is reported as Positive if the mutant:wild-type signal ratio meets or exceeds the clinical cutoff of 0.05.

Method Description

ITD Mutations of *FLT3*

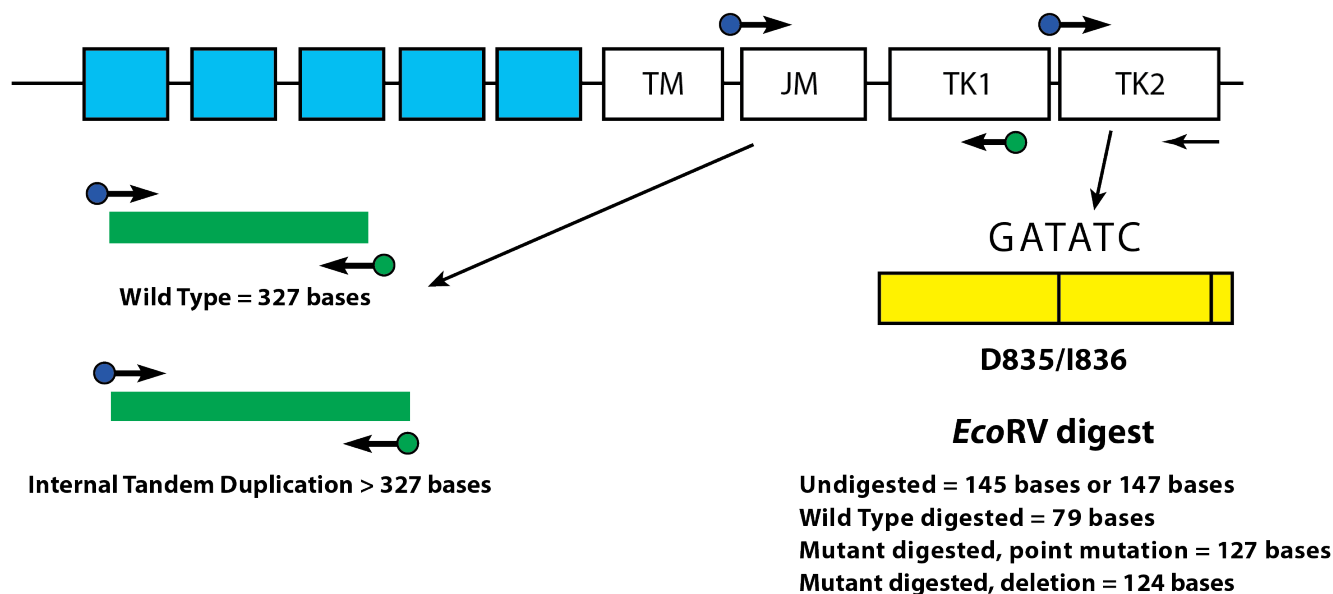
The LeukoStrat CDx *FLT3* Mutation Assay uses fluorescently labeled primers that are in and around the JM region. Wild-type *FLT3* alleles will amplify and produce a product at 327±1 bp as measured by this assay, while alleles that contain ITD mutations will produce a product that exceeds 327±1 bp (see Figure, right).

TKD Mutations of *FLT3*

The LeukoStrat CDx *FLT3* Mutation Assay uses primers that lie on either side of the TKD region. The *FLT3* target region is amplified using PCR and then an EcoRV restriction digest is performed. Wild-type alleles of the *FLT3* gene yield digestion products of 79±1 bp whereas mutant alleles yield products of 125±1 bp or 127±1 bp from the original undigested amplicon product of 145±1 bp or 147±1 bp, as measured by this assay (see Figure, right).

References

1. Murphy KM et al., A Clinical PCR/Capillary Electrophoresis Assay for the Detection of Internal Tandem Duplication and Point Mutation of the *FLT3* Gene. *J. Mol. Diag.* 5:96-102 (2003).
2. Yamamoto, Y., et al., Activating mutation of D835 within the activation loop of *FLT3* in human hematologic malignancies. *Blood*, 97(8):2434-9 (2001).



Depicted is a representation of the *FLT3* juxtamembrane (JM) region (TM = transmembrane) and the activating loop of the tyrosine kinase (TK) domain. Black arrows represent the relative positions of primers that target in and around the JM region for ITD or the activating loop of the kinase domain for TKD. Colored dots represent fluorophores on labeled primers. The yellow box has vertical black lines that represent the position of the *EcoRV* restriction digest sites.

Reagents

Reagent Name	Units in Assay
<i>FLT3</i> Extraction Control	1 x 1800 µL tube
<i>FLT3</i> ITD Master Mix	1 x 1500 µL tube
<i>FLT3</i> TKD Master Mix	1 x 1500 µL tube
<i>FLT3</i> ITD Positive Control	1 x 100 µL tube
<i>FLT3</i> TKD Positive Control	1 x 100 µL tube
<i>FLT3</i> No Template Control	1 x 200 µL tube
Taq DNA Polymerase Enzyme	1 x 200 µL tube
<i>EcoRV</i> Enzyme	1 x 200 µL tube
NEBuffer r3.1	1 x 1250 µL tube

Ordering Information

Catalog #	Products	Quantity
K-412-0331	LeukoStrat® CDx <i>FLT3</i> Mutation Assay (Japan)	33 reactions
K-412-0341	LeukoStrat® CDx <i>FLT3</i> Software (Japan)	1 CD complimentary with purchase

These are *in vitro* diagnostic products, and are available for sale or use within Japan.

Gel and Capillary

RESEARCH USE ONLY (RUO) ASSAYS

B-Cell Assays

- 90 *IGH + IGK* B-Cell Clonality Assays
- 92 *IGH* Gene Rearrangement Assays
- 94 *IGH* Gene Clonality Assays
- 96 *IGK* Gene Clonality Assays
- 98 *IGL* Gene Clonality Assays

T-Cell Assays

- 100 *TCRB* Gene Clonality Assays
- 102 T-Cell Receptor Gama Gene Rearrangement Assays 2.0
- 104 T-Cell Receptor Gama Gene Rearrangement Assays
- 106 *TCRD* Gene Clonality Assays

Translocation Assays

- 108 *BCL1/J_H* Translocation Assay
- 110 *BCL2/J_H* t(14;18) Translocation Assay
- 112 *BCL2/J_H* Translocation Assay
- 114 *BCR/ABL* t(9;22) Translocation Assays
- 116 *PML/RAR α* t(15;17) Translocation Assays

SHM Assays

- 118 *IGH* Somatic Hypermutation Assays v2.0

FLT3 Mutation Assays

- 120 *FLT3* Mutation Assays

Warranty and Liability

Invivoscribe, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe shall have no liability for direct, indirect, consequential or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

Notice

Many of these products are covered by one or more patents including European Patent No. 1549764, European Patent No. 2418287, European Patent No. 2460889, Japanese Patent No. 4708029, United States Patent No. 8859748 and United States Patent No. 10280462. All of these patents are licensed to Invivoscribe®.

These products require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). No license under these patents to use amplification processes or enzymes is conveyed expressly or by implication to the purchaser by the purchase of these products.

Gel and Capillary

Invivoscribe offers an array of assays for B- and T-cell gene clonality/rearrangements, mutations, and chromosome translocations for the study of hematologic malignancies.

These (RUO) assays are available for either ABI capillary electrophoresis fluorescence, or PAGE/agarose gel detection, and contain the PCR master mixes, recommended controls, and Instructions For Use.

On the following pages, you will find detailed information on each RUO assay, including: assay use, background information, typical output data, kit contents, and ordering information. These assays are available in regular sizes (30 or 33 reactions). Select assays are also available in high-volume MegaKit formats (300 or 330 reactions).

These pages contain Research Use Only products which are not for use in diagnostic procedures. Research Use Only (RUO) assays are not for sale in Europe and other global markets where equivalent CE-IVD assays are available and registered with the appropriate regulatory agencies. Refer to the preceding pages for information regarding our IdentiClone® and LeukoStrat® CE-IVD Assays.

For more information, please visit www.invivoscribe.com

IGH + IGK B-Cell Clonality Assays

Assay Use

Immunoglobulin heavy chain (*IGH*) and Kappa light chain (*IGK*) gene clonality assays are useful for identification of B-cell clonality, studying clonal B-cell populations, and evaluation of new research and methods in malignancy studies.

Summary and Explanation of the Test

Five PCR master mixes are included in these test kits to test for rearrangements of both *IGH* and *IGK*. *IGH* Tubes A, B, and C target the conserved framework 1, 2, and 3 regions (respectively) within the variable (V_H) region and the joining (J_H) region of the *IGH* locus. *IGK* tubes A and B target the variable (V_k), intragenic and joining (J_k), and kappa deleting element (K_{del}) regions of the *IGK* locus.

Positive and negative controls, as well as Specimen Control Size Ladder Master Mix are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if any one of the master mixes generates clonal products.

Background

The immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.33, formerly 14q32.3) includes 46–52 functional and 30 nonfunctional variable (V_H), 27 functional diversity (D_H), and 6 functional joining (J_H) gene segments spread over 1250 kilobases.^{1,2} The most frequently used V_H gene segments in normal and malignant B cells belong to V_{H3} , V_{H4} , and V_{H1} families, which together cover 75–95% of V_H usage. The V_H gene segments contain three framework regions (FR) and two complementarity determining regions (CDR). The FRs are characterized by their similarity among the various V_H segments, whereas the CDRs are highly different even within the same V_H family. The CDRs represent the preferred target sequences for somatic hypermutations; however, somatic mutations can also occur in the FRs. Therefore, family-specific primers in the three different FRs were designed to increase the detection rate of clonal *IGH* B-cell populations and decrease the occurrence of false-negative results due to somatic hypermutation in primer binding sites.¹

The human immunoglobulin kappa (*IGK*) light chain locus on the short arm of chromosome 2 (2p11.2) spans 1820 kb. It is made up of 76 variable (V_k) gene segments belonging to seven subgroups, five joining (J_k) gene segments, and one constant (C_k) gene segment. Productive assembly of the kappa gene is successful in about 60% of human B lymphocytes²; however, even when unsuccessful, clonal B cells generally retain the rearranged kappa genes. The V_k segments encode the first 95 N-terminal amino acids. Positions 96–108 are encoded by one of five joining (J_k) gene segments. The constant (C_k) portion of the kappa light chain (amino acids 109–214) is encoded by a single constant (C_k) region separated from the J_k region by an intron.

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA)

References

1. M Hummel et al., *Leukemia* 17: 2266–2272 (2003).
2. AW Langerak et al., *Leukemia* 17: 2272–2275 (2003).
3. EP Rock, PR Sibbald, MM Davis, and YH Chien. *J. Exp. Med.* 179(1): 323–328 (1994).
4. JJM van Dongen et al., *Leukemia* 17: 2257–2317 (2003).

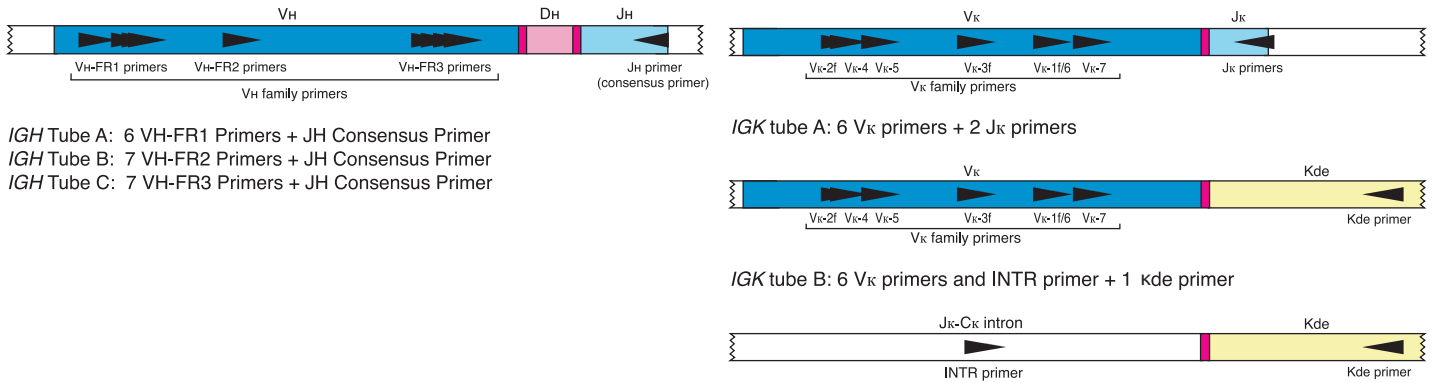


Figure Legend: Simple representation of the organization of a rearranged immunoglobulin heavy chain (*IGH*) gene on chromosome 14 and the immunoglobulin kappa light chain gene on chromosome 2p11.2. Black arrows represent the relative positions of primers that target the conserved framework regions (FR1-3) and the downstream consensus JH gene segments for *IGH* and the V_κ, J_κ, INTR and K_{de} primers which are included in the *IGK* master mix tubes.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0019 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0007 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
<i>IGH</i> Tube A	Framework 1 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
<i>IGH</i> Tube B	Framework 2 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
<i>IGH</i> Tube C	Framework 3 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
<i>IGK</i> Tube A	V _κ -J _κ	1 x 1500 µL tube	10 x 1500 µL tubes
<i>IGK</i> Tube B	V _κ -K _{de}	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
1-100-0010	<i>IGH</i> + <i>IGK</i> B-Cell Clonality Assay - Gel Detection	33 reactions
1-100-0031	<i>IGH</i> + <i>IGK</i> B-Cell Clonality Assay - ABI Fluorescence Detection	33 reactions
1-100-0041	<i>IGH</i> + <i>IGK</i> B-Cell Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

IGH Gene Rearrangement Assays

Assay Use

Immunoglobulin heavy chain (*IGH*) gene clonality assays are useful for the study of identifying clonal B-cell populations and evaluation of new research and methods in malignancy studies.

Summary and Explanation of the Test

Genomic DNA is amplified using three PCR master mixes that target the three conserved framework regions (FR1, FR2, and FR3) of the *IGH* gene and the joining (J_H) region. These regions flank the unique, hypervariable, antigen-binding, complementarity determining region 3 (CDR3). All positive and negative DNA controls, as well as an Amplification Control master mix, are included. PCR products can be analyzed by capillary electrophoresis or standard gel electrophoresis with ethidium bromide staining. Clonality is indicated if one or more of the three framework master mixes generates clonal products.

Background

Genes encoding immunoglobulin heavy chain (*IGH*) molecules are assembled from multiple polymorphic gene segments that undergo rearrangement and selection during B-cell development.² Rearrangement of these variable (V_H), diversity (D_H), and joining (J_H) genetic segments result in VDJ products of unique length and sequence.^{1,2} Clonal *IGH* rearrangements can be rapidly identified through analyses of the size distributions of DNA products amplified from conserved sequences that flank this region.² For example, DNA isolated from a normal polyclonal population of B cells produces a Gaussian distribution (bell-shaped size curve) of amplified products; whereas, DNA amplified from a clonal B-cell population generates one or two product(s) of unique size that reflect proliferation of a single rearranged clone.¹

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

1. JE Miller, SS Wilson, DL Jaye, and M Kronenberg. *J. Mol. Diag.* 4: 101-117 (1999).
2. S Tonegawa. *Nature* 302: 575-581 (1983).

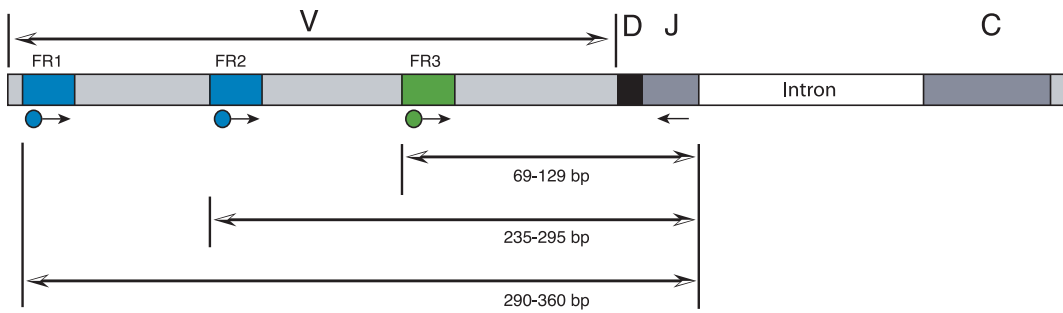


Figure Legend: Genomic organization of a rearranged immunoglobulin heavy chain gene on chromosome 14. The blue and green arrows represent primers targeting the conserved framework regions within the variable region gene. The relative location, size range of valid products, and colors correspond to the products generated from each of these regions when differential fluorescence detection methods are used.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0029 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
<i>IGH</i> Framework 1	Framework 1 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
<i>IGH</i> Framework 2	Framework 2 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
<i>IGH</i> Framework 3	Framework 3 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
Amplification Control	<i>HLA-DQα</i>	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information		
Catalog #	Products	Quantity
1-101-0010	<i>IGH</i> Gene Rearrangement Assay - Gel Detection	30 reactions
1-101-0051	<i>IGH</i> Gene Rearrangement Assay - ABI Fluorescence Detection	30 reactions
1-101-0071	<i>IGH</i> Gene Rearrangement Assay MegaKit - ABI Fluorescence Detection	300 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

IGH Gene Clonality Assays

Assay Use

Immunoglobulin heavy chain (*IGH*) clonality assays are useful for identification of B-cell clonality, studying clonal B-cell populations, and evaluation of new research and methods in malignancy studies.

Summary and Explanation of the Test

Five master mixes target conserved regions within the variable (V_H), diversity (D_H), and the joining (J_H) regions that flank the unique hypervariable, antigen-binding, complementarity determining region 3 (CDR3). Tube A contains six framework region 1 (FR1) primers and a consensus J_H region primer. Tube B contains seven framework region 2 (FR2) primers and a consensus J_H primer. Tube C contains seven framework region 3 (FR3) primers and a consensus J_H primer. Tube D contains six D_H region primers and a consensus J_H region primer. Tube E contains a D_H7 region primer and a consensus J_H primer. Positive and negative controls, as well as the Specimen Control Size Ladder Master Mix are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if any one of the Master mixes generates a clonal product.

Background

The immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.33, formerly 14q32.3) includes 46–52 functional and 30 nonfunctional variable (V_H), 27 functional diversity (D_H), and 6 functional joining (J_H) gene segments spread over 1250 kilobases.^{1,2} The most frequently used V_H gene segments in normal and malignant B cells belong to the V_H3 , V_H4 , and V_H1 family, together covering 75–95% of V_H usage. The V_H gene segments contain three framework regions (FR) and two complementarity determining regions (CDR).

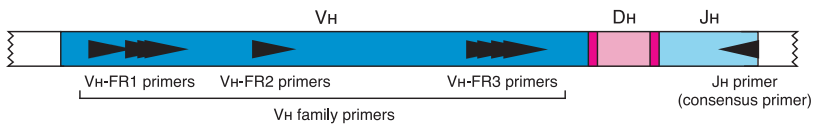
The FRs are characterized by their similarity among the various V_H segments, whereas the CDRs are highly different even within the same V_H family. The CDRs represent the preferred target sequences for somatic hypermutations; however, somatic mutations can also occur in the FRs. Therefore, family-specific primers in the three different FRs were designed to increase the detection rate of clonal *IGH* B-cell populations and decrease the occurrence of false-negative results due to somatic hypermutation in primer binding sites.¹ In addition to V_H - J_H rearrangements, incomplete D_H - J_H rearrangements have been found in mature and immature B-cell malignancies. Therefore, D_H - J_H PCR analysis may be of added value for clonality assessment.²

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

1. M Hummel et al., *Leukemia* 17:2266–2272 (2003).
2. AW Langerak et al., *Leukemia* 17:2272–2275 (2003).
3. JJM van Dongen et al., *Leukemia* 17:2257–2317 (2003).



- Tube A: 6 V_H -FR1 Primers + J_H Consensus Primer
- Tube B: 7 V_H -FR2 Primers + J_H Consensus Primer
- Tube C: 7 V_H -FR3 Primers + J_H Consensus Primer



- Tube D: 6 D_H Primers + J_H Consensus Primer
- Tube E: D_H 7 Primer + J_H Consensus Primer

Figure Legend: Simple representation of the organization of a rearranged immunoglobulin heavy chain gene on chromosome 14. Black arrows represent the relative positions of primers that target the conserved framework (FR1-3) and diversity (D_H1-7) regions, and the downstream consensus J_H gene segments. The amplicon products generated from each of these regions can be differentially detected when fluorescent primer sets are used with capillary electrophoresis instruments that employ differential fluorescence detection.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0019 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0024 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0008 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
IGH Tube A	Framework 1 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube B	Framework 2 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube C	Framework 3 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube D	D _H 1-6 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube E	D _H 7 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
1-101-0020	IGH Gene Clonality Assay - Gel Detection	33 reactions
1-101-0040	IGH Gene Clonality Assay MegaKit - Gel Detection	330 reactions
1-101-0061	IGH Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
1-101-0081	IGH Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

IGK Gene Clonality Assays

Assay Use

Immunoglobulin kappa light chain (*IGK*) gene clonality assays are useful for the identification of B-cell clonality, studying clonal B-cell populations, and evaluation of new research and methods in malignancy studies.

Summary and Explanation of the Test

Two master mixes target conserved regions within the variable (*Vk*1-7) and the joining (*Jk*1-5) regions that flank the unique hypervariable, antigen-binding, complementarity determining region 3 (CDR3). Other primers target the *K_{de}* and intragenic regions.

Tube A contains six upstream primers and two *Jk* region primers. Tube B contains six upstream *Vk* region primers, an upstream intragenic primer and a downstream *K_{de}* primer. Positive and negative controls, as well as a Specimen Control Size Ladder Master Mix, are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if any one of the master mixes generates clonal products.

Background

The human immunoglobulin kappa (*IGK*) light chain locus on the short arm of chromosome 2 (2p12, formerly 2p11.2) spans 1820 kb. It is made up of 76 variable (*Vk*) gene segments belonging to 7 subgroups, 5 joining (*Jk*) gene segments, and one constant (*Ck*) gene segment. Productive assembly of the kappa gene is successful in about 60% of human B lymphocytes.¹ However, even when unsuccessful, clonal B cells generally retain the rearranged kappa genes. The *Vk* segments encode the first 95 N-terminal amino acids. Positions 96-108 are encoded by one of five joining (*Jk*) gene segments. The constant (*Ck*) portion of the kappa light chain (amino acids 109-214) is encoded by a single constant (*Ck*) region separated from the *Jk* region by an intron. The length of the hypervariable complementarity determining region 3 (CDR3) in kappa light chain genes is limited and rearrangements in this region display significant skewing (platykurtosis).²

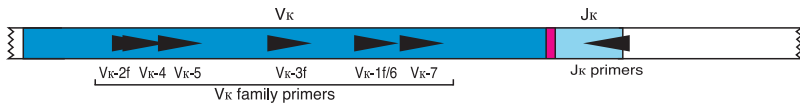
Therefore, clonal CDR3 products generated from this region are most easily and reliably identified by heteroduplex analysis using standard polyacrylamide gels. Alternatively, capillary electrophoresis or gene sequencing instruments coupled with differential fluorescence detection can be used for analysis.

Specimen Requirements

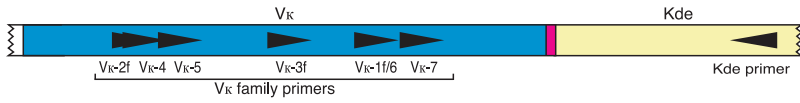
This assay tests extracted and purified genomic DNA (gDNA).

References

1. AW Langerak et al., *Leukemia* 17: 2275-2280 (2003).
2. EP Rock, PR Sibbald, MM Davis, and YH Chien. *J. Exp. Med.* 179(1): 323-328 (1994).
3. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).



IGK tube A: 6 V_{κ} primers + 2 J_{κ} primers



IGK tube B: 6 V_{κ} primers and INTR primer + 1 K_{de} primer

Figure Legend: Schematic diagram of the immunoglobulin kappa light chain gene complex on chromosome 2p11.2. Shown are the relative positions and orientations for the V_{κ} - J_{κ} , and K_{de} primers, which are included in the *IGK* master mix tubes.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0007 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
<i>IGK</i> Tube A	V_{κ} - J_{κ}	1 x 1500 µL tube	10 x 1500 µL tubes
<i>IGK</i> Tube B	V_{κ} - K_{de}	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
1-102-0020	<i>IGK</i> Gene Clonality Assay - Gel Detection	33 reactions
1-102-0030	<i>IGK</i> Gene Clonality Assay MegaKit - Gel Detection	330 reactions
1-102-0021	<i>IGK</i> Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
1-102-0031	<i>IGK</i> Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

IGL Gene Clonality Assays

Assay Use

Immunoglobulin lambda light chain (*IGL*) gene clonality assays are useful for the identification of B-cell clonality, studying clonal B-cell populations, and evaluation of new research and methods in malignancy studies.

Summary and Explanation of the Test

The *IGL* Tube master mix targets conserved regions within the variable ($V_{\lambda 1-3}$) and the joining ($J_{\lambda 1-3}$) regions that flank the unique, hypervariable, antigen-binding, complementarity determining region 3 (CDR3). Positive and negative controls, as well as a Specimen Control Size Ladder Master Mix, are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if the master mix generates clonal products.

Background

The human immunoglobulin lambda (*IGL*) light chain locus is located on the long arm of chromosome 22 (22q11.2) and spans 1050 kilobases. It is made up of 73-74 variable (V_{λ}) gene segments (spread over 900 kilobases), 7-11 joining (J_{λ}) gene segments and 7-11 constant (C_{λ}) gene segments depending on the haplotypes. Of the 73-74 V_{λ} region genes, only 30-33 are functional and can be grouped into 11 families and 3 clans.¹ The J_{λ} and C_{λ} region genes are organized in tandem with a J_{λ} segment preceding a C_{λ} gene. Typically there are 7 J_{λ} - C_{λ} segments of which four are functional and encode the four Ig lambda isotypes.

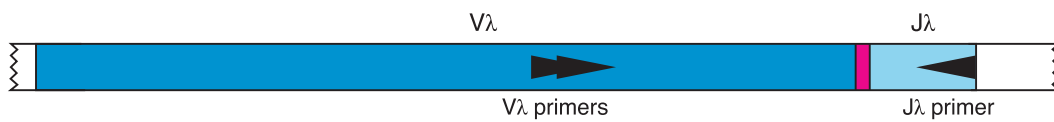
IGL gene rearrangements (V_{λ} - J_{λ}) rearrangements potentially represent an attractive PCR target for clonality studies to compensate for false-negative *IGH* V_H - J_H PCR results mainly caused by somatic hypermutations. The limited size of the junctional region may create a challenge to distinguish polyclonal from monoclonal rearrangements when running a simple agarose or polyacrylamide gel.¹ Therefore, clonal V_{λ} - J_{λ} PCR products are most easily and reliably identified by heteroduplex analysis using standard polyacrylamide gels. Alternatively, capillary electrophoresis or gene sequencing instruments coupled with differential fluorescence detection can also be used for analysis.¹

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

1. F Davi et al., *Leukemia* 17:2280-2283 (2003).
2. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).



***IGL* tube: 2 $V\lambda$ primers + 1 $J\lambda$ primer**

Figure Legend: Schematic diagram of the immunoglobulin lambda light chain gene complex on chromosome 22q11.2. Shown are the relative positions and orientations for the $V\lambda$ and $J\lambda$ primers, which are included in the *IGL* master mix tube. The two $V\lambda$ primers only target $V\lambda 1, 2,$ and 3 because these three V families cover approximately 70% of rearrangeable $V\lambda$ gene segments, and approximately 90% of all *IGL* gene rearrangements involve these three families. Similarly, the single $J\lambda$ primer only targets $J\lambda 1, 2,$ and 3 because these three J segments are involved in 98% of all *IGL* gene rearrangements.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0010 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0029 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
<i>IGL</i> Tube	$V\lambda$ - $J\lambda$	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
1-103-0010	<i>IGL</i> Gene Clonality Assay - Gel Detection	33 reactions
1-103-0020	<i>IGL</i> Gene Clonality Assay MegaKit - Gel Detection	330 reactions
1-103-0011	<i>IGL</i> Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
1-103-0021	<i>IGL</i> Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

TCRB Gene Clonality Assays

Assay Use

T-Cell receptor beta (*TCRB*) gene clonality assays are useful for the identification of T-cell clonality, studying clonal T-cell populations, and evaluation of new research and methods in malignancy studies.

Summary and Explanation of the Test

Three multiplex master mixes target conserved regions within the variable ($V\beta$), diversity ($D\beta$), and the joining ($J\beta$) regions that flank the unique hypervariable, antigen-binding, complementarity determining region 3 (CDR3) of the T-cell receptor beta locus. Tube A contains 23 $V\beta$ primers, six $J\beta 1$ primers, and three $J\beta 2$ primers. Tube B contains 23 $V\beta$ and four $J\beta 2$ primers. Tube C contains two $D\beta$ and 13 $J\beta$ primers. Positive and negative DNA controls, as well as a Specimen Control Size Ladder Master Mix, are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated, if any one of the master mixes generates clonal products.

Background

The human T-cell receptor beta (*TRB*, formerly known as *TCRB*) gene locus on chromosome 7 (7q34, formerly 7q35) includes 64–67 variable ($V\beta$) gene segments (belonging to 30 subgroups), two diversity ($D\beta$) gene segments, and 13 joining ($J\beta$) gene segments, spread over 685 kilobases. The diversity of this locus has complicated PCR-based testing and extended dependence on Southern blot analysis in many testing centers. However, this standardized multiplex PCR assay detects the vast majority of clonal *TRB* gene rearrangements using only three multiplex master mixes.¹

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

1. M Brüggemann et al., *Leukemia* 17: 2283–2289 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257–2317 (2003).



Tube A: 23 Vβ primers + 6 Jβ1 primers and 3 Jβ2 primers

Tube B: 23 Vβ primers + 4 Jβ2 primers

Tube C: 2 Dβ primers + 13 Jβ primers

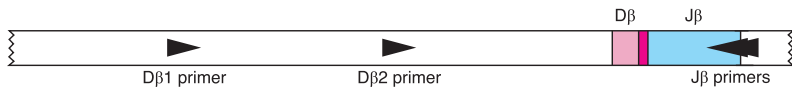


Figure Legend: Simplified diagram of a representative rearranged T-cell receptor beta gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for Master Mix Tubes A, B, and C.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0009 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0004 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0021 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
TCRB Tube A	Multiple Vβ + Jβ1/2	1 x 1500 µL tube	10 x 1500 µL tubes
TCRB Tube B	Multiple Vβ + Jβ2	1 x 1500 µL tube	10 x 1500 µL tubes
TCRB Tube C	Multiple Dβ + Jβ1/2	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
1-205-0010	TCRB Gene Clonality Assay - Gel Detection	33 reactions
1-205-0020	TCRB Gene Clonality Assay MegaKit - Gel Detection	330 reactions
1-205-0011	TCRB Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
1-205-0021	TCRB Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

T-Cell Receptor Gamma Gene Rearrangement Assay 2.0

Assay Use

This Research Use Only assay identifies T-cell receptor gamma (*TCRG*) chain gene rearrangements and is useful for the identification of clonal T-cell populations and evaluation of new research and methods in malignancy studies.

Summary and Explanation of the Test

This T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 contains a single multiplex master mix which includes primers for all known groups of *TCR* gamma variable (*Vy*) region genes and joining (*Jy*) region genes involved in rearrangements. In addition, positive and negative controls, as well as a Specimen Control Size Ladder Master Mix are included. PCR products can be analyzed by polyacrylamide or capillary electrophoresis detection.

Background

The human T-cell receptor gamma (*TRG*, formerly known as *TCRG*) gene locus on chromosome 7 (7q14) includes 14 *Vy* genes belonging to four subgroups, five *Jy* segments, and two *Cy* genes spread over 200 kilobases. The diversity of this locus has historically complicated PCR-based testing. This multiplex PCR assay represents an improvement over other assays as it can detect the vast majority of *TCR* gamma gene rearrangements with a single multiplex master mix. This master mix targets all conserved regions within the variable (*Vy*) and joining (*Jy*) region genes, providing a more comprehensive analysis to include *Vy* and *Jy* regions that would not be identified with a single *Vy* (1-8) and *Jy* 1/*Jy* 2 primer set.

In addition, competitive amplification of all *TRG* gene rearrangements allows for identification of a quantitative threshold for a positive result and helps to avoid false positive results. The average size of the *TRG* gene rearrangement PCR amplicons is 190 nucleotides, with a normal distribution of product sizes between 159 and 207 nucleotides. This protocol leads to improved product formation from formalin-fixed, paraffin-embedded (FFPE) samples compared to other protocols that yield products of 260 nucleotides or larger.

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

1. TC Greiner et al., *JMD* 4: 137-143 (2002).
2. LC Lawnickie et al., *JMD* 5: 82-87 (2003).
3. Y Sandberg et al., *Leukemia* 21: 21 (2007).
4. Armand, Marine et al. *HemaSphere*, 2019;3:3.

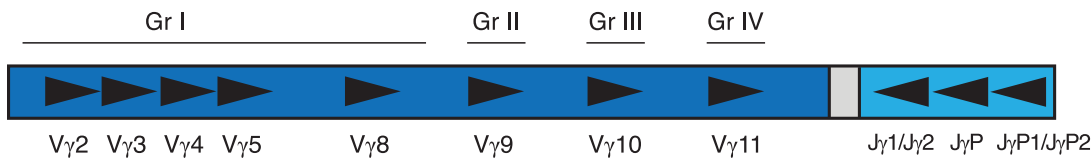


Figure Legend: Simple representation of the organization of the T-cell receptor gamma gene on chromosome 7. Black arrows represent the relative positions of primers that target the variable region genes and the downstream joining region gene segments that are involved in rearrangements in T-cell lymphomas. The downstream primers are fluorescently labeled through the incorporation of a 6FAM fluorophore. The amplicon products generated from these rearrangements are detected by capillary electrophoresis.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
5% <i>TCRG</i> Positive Control DNA	50 µg/mL	1 x 50 µL tube	5 x 50 µL tube
<i>TCRG</i> Negative Control DNA	50 µg/mL	1 x 50 µL tube	5 x 50 µL tube
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
<i>TCRG</i> - 6FAM	V γ 1-V γ 11 + J γ 1/J γ 2, J γ P, J γ P1/J γ P2	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
1-207-0101	T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 - ABI Fluorescence Detection	33 reactions
1-207-0111	T-Cell Receptor Gamma Gene Rearrangement Assay MegaKit 2.0 - ABI Fluorescence Detection	330 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

T-Cell Receptor Gamma Gene Rearrangement Assays

Assay Use

T-Cell receptor gamma (*TCRG*) gene clonality assays are useful for the identification of T-cell clonality, studying clonal T-cell populations, and evaluation of new research and methods in malignancy studies.

Summary and Explanation of the Test

Sample genomic DNA is amplified using two master mixes that independently target conserved regions within the variable (*V γ*) and joining (*J γ*) regions that flank the unique, hypervariable, antigen-binding, complementarity determining region 3 (CDR3). This assay targets *V γ 1-9* and *J γ* gene segments. Positive and negative DNA controls, as well as an internal Amplification Control Master Mix, are included. PCR products can be analyzed by capillary electrophoresis or standard gel electrophoresis with ethidium bromide staining.

Background

The T-cell receptor gamma (*TRG*, formerly known as *TCRG*) chain locus spans 160 kilobases on chromosome 7 (7p14). The locus consists of 14 variable (*V γ*) gene segments in six subgroups, and five joining (*J γ*) gene segments interspersed between two constant (*C γ*) gene segments. However, the repertoire of functional *TRG* molecules is limited to 4-6 functional *V γ* gene segments that belong to two subgroups.²

Rearrangement of the *V γ* and *J γ* gene segments of the *TRG* locus results in *V γ -J γ* products of unique length and sequence. Clonal *TRG* rearrangements can be most rapidly identified by analyzing the size distribution of DNA products amplified from conserved sequences that flank this *V γ -J γ* region.¹ DNA isolated from a normal heterogeneous population of polyclonal T-cells produces a Gaussian distribution (bell-shaped size curve) of amplified products. DNA amplified from a clonal T-cell population generates one or two product(s) of unique size that reflects proliferation of a single rearranged clone.^{1,2}

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

1. JE Miller, SS Wilson, DL Jaye, and M Kronenberg. *J. Mol. Diag.* 4: 101-117 (1999).
2. K Beldjord et al., *Leukemia* 17: 2289-2292 (2003).

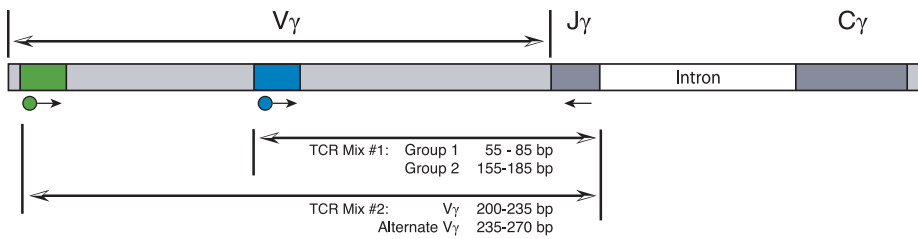


Figure Legend: Simplified figure representing the organization of a rearranged T-cell receptor gamma chain gene on chromosome 7. Colored arrows represent conserved regions within the variable region gene segments targeted by primers. Primers are represented by arrows with the size range of valid products generated with each of the master mixes indicated below the figure. Colors correspond to the peak colors assigned to products when differential fluorescence detection methods are used.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0009 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
T-Cell Receptor Gamma Mix 1	Vy1-8,9 + Jy1/2	1 x 1500 µL tube	10 x 1500 µL tubes
T-Cell Receptor Gamma Mix 2	Alt Vy + Jy1/2	1 x 1500 µL tube	10 x 1500 µL tubes
Amplification Control	<i>HLA-DQα</i>	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
1-207-0010	T-Cell Receptor Gene Rearrangement Assay - Gel Detection	30 reactions
1-207-0051	T-Cell Receptor Gene Rearrangement Assay - ABI Fluorescence Detection	30 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

TCRD Gene Clonality Assays

Assay Use

T-Cell receptor delta (*TCRD*) gene clonality assays are useful for the identification of T-cell clonality, studying clonal T-cell populations, and evaluation of new research and methods in malignancy studies.

Summary and Explanation of the Test

The *TCRD* Tube Master Mix targets conserved regions within the variable ($V\delta 1-6$), the diversity ($D\delta 2-3$) and the joining ($J\delta 1-4$) regions that flank the unique, hypervariable, antigen-binding, complementarity determining region 3 (CDR3) of the T-cell receptor delta (*TRD*, formerly known as *TCRD*). Positive and negative controls, as well as a Specimen Control Size Ladder Master Mix, are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if the master mix generates clonal products.

Background

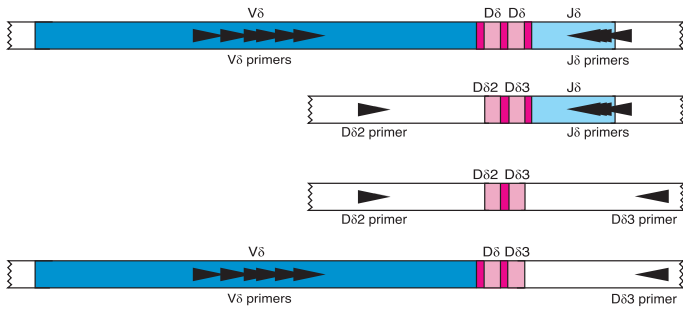
The human T-cell receptor delta (*TRD*, formerly known as *TCRD*) gene locus is comprised of a cluster of 10 genes located on chromosome 14 (14q11.2) spread over 60 kilobases, localized between the T-cell receptor alpha (*TRA*, formerly known as *TCRA*) variable ($V\alpha$) and joining ($J\alpha$) gene segments. It is made up of eight variable ($V\delta$), three diversity ($D\delta$), and four joining ($J\delta$) gene segments.¹ At least five of the eight $V\delta$ gene segments can also rearrange to $J\delta$ gene segments and other $V\delta$ gene segments may also be utilized in *TRD* gene rearrangements in rare cases. Although the small number of $V\delta$, $D\delta$, and $J\delta$ gene segments available for recombination limits the potential combinatorial diversity, the complementarity determining region 3 (CDR3) or junctional diversity is extensive due to the addition of N regions, P regions, and random deletion of nucleotides by recombinases. This diversity is also extended by the recombination of up to three $D\delta$ segments and therefore up to four N regions within the rearranged *TRD* locus. This limited germline diversity encoded at the *TRD* locus in conjunction with extensive junctional diversity results in a useful target for PCR analysis. *TRD* recombination events have been used most extensively as clonal markers in both T- and B-cell ALL. This standardized multiplex PCR assay detects the vast majority of clonal *TRD* gene rearrangements using a single multiplex master mix¹.

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

1. FL Lavender et al., *Leukemia* 17: 2292-2296 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).



TCRD tube: 6 V δ and 1 D δ 2 primers + 4 J δ and 1 D δ 3 primers

Figure Legend: Simplified diagram of a representative rearranged T-cell receptor delta gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for the *TRD* Tube Master Mix tube.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0021 Clonal Control DNA	200 μ g/mL	1 x 100 μ L tube	5 x 100 μ L tubes
IVS-0000 Polyclonal Control DNA	200 μ g/mL	1 x 100 μ L tube	5 x 100 μ L tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
<i>TCRD</i> Tube	Multiple V δ + D δ + J δ	1 x 1500 μ L tube	10 x 1500 μ L tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 μ L tube	10 x 1500 μ L tubes

Ordering Information

Catalog #	Products	Quantity
1-206-0010	<i>TCRD</i> Gene Clonality Assay - Gel Detection	33 reactions
1-206-0020	<i>TCRD</i> Gene Clonality Assay MegaKit - Gel Detection	330 reactions
1-206-0011	<i>TCRD</i> Gene Clonality Assay - ABI Fluorescence Detection	33 reactions
1-206-0021	<i>TCRD</i> Gene Clonality Assay MegaKit - ABI Fluorescence Detection	330 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

BCL1/*J_H* Translocation Assay

Assay Use

These assays identify *BCL1*/*J_H* t(11;14) translocations and are useful for the evaluation of new research and methods in malignancy studies.

Summary and Explanation of the Test

Two master mixes are included in this assay kit. The *BCL1*/*J_H* Master Mix targets the major translocation cluster (MTC) of the *CCND1* locus (formerly known as *BCL1*) and the joining region (*J_H*) of the immunoglobulin heavy chain locus (*IGH*). The Specimen Control Size Ladder Master Mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. Positive and negative controls are included. PCR products can be analyzed using standard gel electrophoresis with ethidium bromide staining. A *CCND1* translocation is indicated if the master mix generates product(s) within the valid size range.

Background

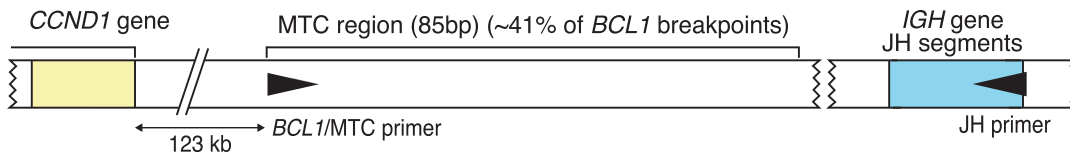
This aberrant *BCL1*/*J_H* t(11;14) translocation juxtaposes genes of the immunoglobulin heavy chain (*IGH*) gene on chromosome 14q32 with the cyclin D1 gene on chromosome 11q13. The juxtaposition of *IGH*-sequences results in the transcriptional activation of cyclin D1.^{2,3} Cyclin D1 is involved in the regulation of the G1 progression and G1/S transition of the cell cycle.³ Translocation does not lead to expression of a fusion protein. In fact, oncogenesis is due to a promoter/enhancer exchange, wherein the immunoglobulin gene enhancer stimulates the expression of cyclin D1. Overexpression of cyclin D1, in turn, accelerates passage of transformed cells through the G1 phase.

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

1. P Wijers et al., *Leukemia* 17: 2296–2298 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257–2317 (2003).
3. Shimazaki C, et. al., (1997). *International Journal of Hematology*. 66(1):111–5.



t(11;14) tube: 1 *BCL1* MTC primer + 1 JH primer

Figure Legend: Schematic diagram of the *IGH-CCND1* t(11;14) translocation showing the cyclin D1 (*CCND1*) gene on the left and the Ig heavy chain (*IGH*) gene on the right. Shown are the relative positions and orientations for the *BCL1*/MTC primer and the J_H primer, which are included in the *BCL1*/J_H Master Mix tube.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0010 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
<i>BCL1</i> /J _H Tube	MTC of <i>CCND1</i> + <i>IGH</i> J _H	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
1-308-0010	<i>BCL1</i> /J _H Translocation Assay - Gel Detection	33 reactions
1-308-0020	<i>BCL1</i> /J _H Translocation Assay MegaKit - Gel Detection	330 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

BCL2/*J_H* t(14;18) Translocation Assays

Assay Use

This Research Use Only assay identifies *BCL2*/*J_H* t(14;18) translocations.

Summary and Explanation of the Test

Five master mixes are included in this assay kit. Two master mixes target *BCL2* major break point (MBR) translocations and two target *BCL2* minor cluster region (mcr) translocations. An Amplification Control Master Mix is also included to ensure the quality and quantity of sample DNA. Positive and negative controls are also included. This assay can be run either in a standard or nested assay format. PCR products can be analyzed by standard gel electrophoresis with ethidium bromide staining. A *BCL2* translocation is indicated if just one of the 2nd round master mixes (mixes ending in b) generates product(s) within the valid size range.

Background

BCL2 translocations are reciprocal chromosome exchanges that place the *bcl-2* proto-oncogene, located on chromosome 18, under aberrant transcriptional control of the immunoglobulin heavy chain gene, located on chromosome 14. The *bcl-2* protein is an antagonist to apoptosis (programmed cell death), a normal process designed to eliminate unneeded and damaged cells during hematopoiesis. Increased expression of the *bcl-2* protein leads to an increase in the levels of B-cells in the body.

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

1. MS Lee et al., *Science* 237: 175-178 (1987).
2. M Crescenzi et al., *Proc. Natl. Acad. Sci. USA* 85: 4869-4873 (1988).

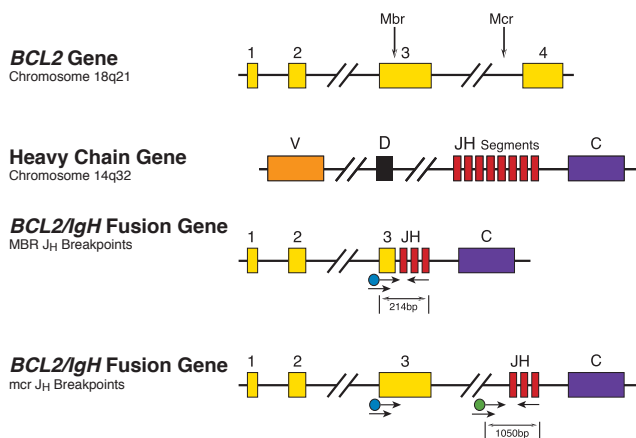


Figure Legend: Simplified view of the genomic organization of the *BCL2* and *IGH* genes on chromosomes 18 and 14, respectively. Yellow boxes represent the exon regions of the *BCL2* gene. Exons of the immunoglobulin heavy chain gene are represented in other colors. The solid black lines represent intron regions, which have been left incompletely spliced to assist in demarcation of the exon segments. MBR and mcr type t(14;18) translocations of the exon segments. MBR and mcr type t(14;18) translocations are shown in the lower portions of the figure with the relative positions of primers and the size of the amplicons generated from the positive control DNAs indicated.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0031 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0009 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
<i>BCL2</i> / <i>JH</i> t(14;18) (MBR) Mix 1b	Inside <i>BCL2</i> MBR	1 x 1500 µL tube	10 x 1500 µL tubes
<i>BCL2</i> / <i>JH</i> t(14;18) (mcr) Mix 2b	Inside <i>BCL2</i> mcr	1 x 1500 µL tube	10 x 1500 µL tubes
<i>BCL2</i> / <i>JH</i> t(14;18) (MBR) Mix 1a	Outside <i>BCL2</i> MBR	1 x 1500 µL tube	10 x 1500 µL tubes
<i>BCL2</i> / <i>JH</i> t(14;18) (mcr) Mix 2a	Outside <i>BCL2</i> mcr	1 x 1500 µL tube	10 x 1500 µL tubes
Amplification Control	<i>HLA-DQa</i>	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
1-309-0010	<i>BCL2</i> / <i>JH</i> t(14;18) Translocation Assay - Gel Detection	30 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

BCL2/J_H Translocation Assay

Assay Use

This Research Use Only assay identifies BCL2/J_H translocations.

Summary and Explanation of the Test

Four master mixes are included in this assay. Three are used to identify translocations in the major breakpoint region (MBR) and minor cluster region (mcr) of BCL2. The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. This assay includes negative control DNA and positive control DNAs for both the MBR and mcr. PCR products can be analyzed using standard gel electrophoresis with ethidium bromide staining. A BCL2 translocation is indicated if any one of the master mixes generates product(s) within the valid size range.

Background

BCL2 translocations are reciprocal chromosome exchanges that place the *bcl-2* proto-oncogene, located on chromosome 18, under aberrant transcriptional control of the immunoglobulin heavy chain gene, located on chromosome 14. The *bcl-2* protein is an antagonist to apoptosis (programmed cell death), a normal process designed to eliminate unneeded and damaged cells during hematopoiesis. Increased expression of the *bcl-2* protein leads to an increase in the levels of B-cells in the body.

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

1. PAS Evans et al., *Leukemia* 17: 2298-2301 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).



t(14;18) tube A: 2 BCL2 MBR primers + 1 J_H primer

t(14;18) tube B: 4 BCL2 3'MBR primers + 1 J_H primer

t(14;18) tube C: 3 BCL2 mcr primers + 1 J_H primer

Figure Legend: Schematic diagram of the IGH-BCL2 t(14;18) translocation showing the BCL2 gene on the left and the Ig heavy chain (IGH) gene on the right. Shown are the relative positions and orientations for the major breakpoint region (MBR) primers, the minor cluster region (mcr) primers, and the J_H primer, which are included in the 3 BCL2/J_H master mix tubes.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-P002 Clonal Control DNA	1600 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0031 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
<i>BCL2</i> / <i>JH</i> Tube A	<i>BCL2</i> MBR + <i>IGH</i> <i>JH</i>	1 x 1500 µL tube	10 x 1500 µL tubes
<i>BCL2</i> / <i>JH</i> Tube B	<i>BCL2</i> 3' MBR + <i>IGH</i> <i>JH</i>	1 x 1500 µL tube	10 x 1500 µL tubes
<i>BCL2</i> / <i>JH</i> Tube C	<i>BCL2</i> mcr + <i>IGH</i> <i>JH</i>	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
1-309-0020	<i>BCL2</i> / <i>JH</i> Translocation Assay - Gel Detection	33 reactions
1-309-0040	<i>BCL2</i> / <i>JH</i> Translocation Assay MegaKit - Gel Detection	330 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

BCR/ABL t(9;22) Translocation Assays

Assay Use

This Research Use Only assay identifies *BCR/ABL* t(9;22) translocations.

Summary and Explanation of the Test

The master mixes are included in these assay kits used to amplify complementary DNA (cDNA) produced from specimen(s), and positive and negative RNA controls (included). Primers target an internal control transcript (*Abl*) and p190-, p210-, and p230-type transcripts expressed from *BCR-ABL1* translocations. Amplicon products can be analyzed by capillary electrophoresis or standard gel electrophoresis with ethidium bromide staining. A *BCR-ABL1* translocation is indicated if just one of the 2nd round master mixes (Mix 2b, Mix 2c, Mix 3b, Mix 3c, or Mix 3d) generates product(s) of the valid size. Reagents for RNA extraction and reverse transcription are not included. This assay is compatible with all standard RNA extraction and cDNA synthesis methods. This is a qualitative assay and has not been validated for quantitative use.

Background

BCR/ABL translocations are associated with a variety of hematologic malignancies. The Philadelphia chromosome (Ph1) is a specific chromosomal abnormality that results from reciprocal t(9;22)(q34;q11) chromosomal rearrangements fuse coding regions of the *BCR* gene, located on chromosome 22, with the *ABL* receptor-independent tyrosine kinase gene on chromosome 9. This assay detects and identifies the variety of p190-, p210- and p230-type transcripts produced from all known *BCR/ABL* translocations.

Specimen Requirements

This assay tests complementary DNA (cDNA) template.

References

1. R Kurzrock et al., *Ann. Intern. Med.* 138: 819-30 (2003).
2. JV Melo. *Blood* 88: 2375-2384 (1996).
3. JP Radich et al., *Blood* 85: 2632-2638 (1995).

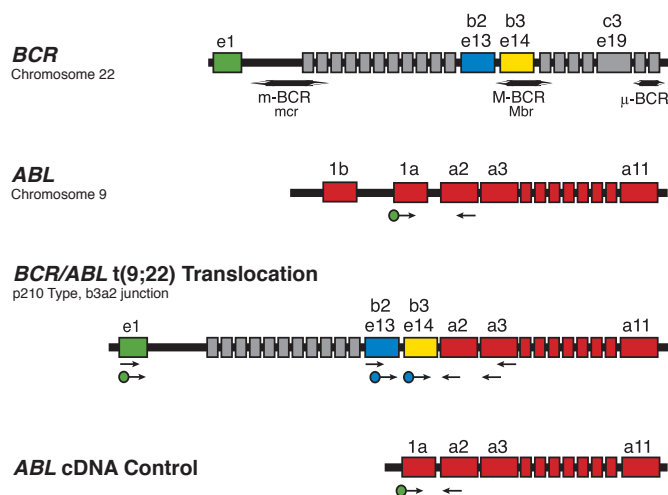


Figure Legend: This figure shows the genomic organization of the *BCR* and *ABL* genes on chromosomes 22 and 9, respectively. Boxes represent exon regions of the *ABL* (red boxes) and *BCR* encoding exons (other colors). The solid black line represents intron regions, which have been left incompletely spliced to assist in demarcation of the exon segments. The location of exon regions targeted by labeled and unlabeled primers are indicated by arrows. A p210-type *BCR-ABL1* translocation (b3a2 junction) is depicted in the lower portion of the figure along with the control *ABL* transcript control.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0032 Clonal Control RNA	400 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0011 Clonal Control RNA	400 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0035 Clonal Control RNA	400 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
<i>BCR/ABL</i> t(9;22) Mix 1a	Abl	1 x 1500 µL tube	10 x 1500 µL tubes
<i>BCR/ABL</i> t(9;22) Mix 2a	p190	1 x 1500 µL tube	10 x 1500 µL tubes
<i>BCR/ABL</i> t(9;22) Mix 3a	p210+230	1 x 1500 µL tube	10 x 1500 µL tubes
<i>BCR/ABL</i> t(9;22) Mix 1b	Abl	1 x 1500 µL tube	10 x 1500 µL tubes
<i>BCR/ABL</i> t(9;22) Mix 2b	p190	1 x 1500 µL tube	10 x 1500 µL tubes
<i>BCR/ABL</i> t(9;22) Mix 2c	p190	1 x 1500 µL tube	10 x 1500 µL tubes
<i>BCR/ABL</i> t(9;22) Mix 3b	p210+230	1 x 1500 µL tube	10 x 1500 µL tubes
<i>BCR/ABL</i> t(9;22) Mix 3c	p210+230	1 x 1500 µL tube	10 x 1500 µL tubes
<i>BCR/ABL</i> t(9;22) Mix 3d	p210+230	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
1-310-0010	<i>BCR/ABL</i> t(9;22) Translocation Assay – Gel Detection	30 reactions
1-310-0031	<i>BCR/ABL</i> t(9;22) Translocation Assay – ABI Fluorescence Detection	30 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

PML/RAR α t(9;22) Translocation Assays

Assay Use

This Research Use Only assay identifies PML/RAR α t(15;17) translocations.

Summary and Explanation of the Test

Four master mixes are included in these assay kits. Master mixes are used to amplify complementary DNA (cDNA) produced from specimen(s), as well as positive and negative RNA controls (included). Primers target an internal control transcript and the variety of Bcr1, Bcr2, and Bcr3 type transcripts expressed from PML-RAR α translocations. Amplicon products can be analyzed by differential fluorescence detection using capillary electrophoresis or standard gel electrophoresis. A PML-RAR α translocation is indicated if just one of the 2nd round master mixes (Mix 2b or Mix 2c) generates product(s) of the valid size. Reagents for RNA extraction and reverse transcription are not included. This assay is compatible with all standard RNA extraction and cDNA synthesis methods. This is a qualitative assay and has not been validated for quantitative use.

Background

Three PML/RAR α translocation patterns have been identified in samples with acute myelogenous leukemia (AML): type A is the short (S-form); the breakpoint occurs within breakpoint cluster region 3 (Bcr-3). Type B is the long (L-form); the breakpoint occurs within Bcr-1. There is a third type B variant or variable (V-form); the breakpoint is within Bcr-2. Identification of the PML/RAR α t(15;17) rearrangements are commonly used in the study of APL because it is correlated with responsiveness to treatment. This RT-PCR method directly identifies the chimeric PML/RAR α transcripts expressed from all three forms of PML/RAR α translocations.

Specimen Requirements

This assay tests complementary DNA (cDNA) template.

References

1. H De Thé et al., *Nature* 347: 558-561 (1990).
2. H De Thé et al., *Cell* 66: 675-684 (1991).
3. A Kakizuka et al., *Cell* 66: 663-674 (1991).
4. WH Miller et al., *Proc. Natl. Acad. Sci.* 89: 2694-2698 (1992).

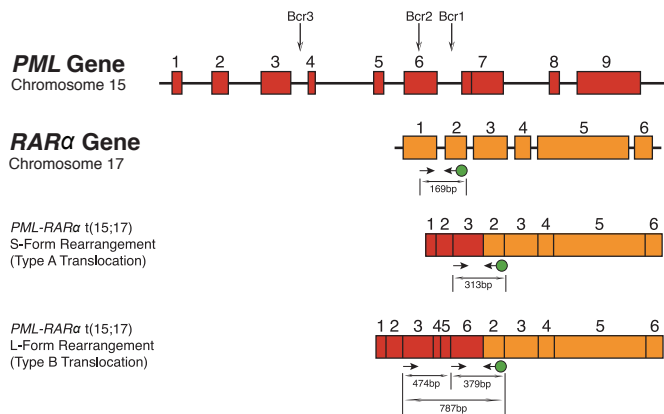


Figure Legend: This figure shows the genomic organization of the PML and RAR α genes on chromosomes 15 and 17, respectively. Boxes represent exon regions of the PML (red boxes) and RAR α (orange) encoding exons. The solid black line represents intron regions, which were left incompletely spliced to assist in demarcation of the exon segments. Primers are indicated by arrows, and the size of several of the products are indicated below the translocated gene segments. S-form (Bcr3) and L-form (Bcr1) PML-RAR α translocations are depicted in the lower portion of the figure.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0020 Clonal Control RNA	400 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0035 Clonal Control RNA	400 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
<i>PML/RARα</i> t(15;17) Mix 1	<i>RARA</i>	1 x 1500 µL tube	10 x 1500 µL tubes
<i>PML/RARα</i> t(15;17) Mix 2a	<i>PML-RARA</i>	1 x 1500 µL tube	10 x 1500 µL tubes
<i>PML/RARα</i> t(15;17) Mix 2b	S- and L-Forms	1 x 1500 µL tube	10 x 1500 µL tubes
<i>PML/RARα</i> t(15;17) Mix 2c	L-Form	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
1-311-0010	<i>PML/RARα</i> t(15;17) Translocation Assay - Gel Detection	30 reactions
1-311-0011	<i>PML/RARα</i> t(15;17) Translocation Assay - ABI Fluorescence Detection	30 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

IGH Somatic HyperMutation Assays v2.0

Assay Use

The Research Use Only *IGH* Somatic Hypermutation Assay v2.0 is used to identify clonal rearrangements of the immunoglobulin heavy (*IGH*) chain gene and determine the somatic mutation status of the variable (V) gene sequence and is useful for the study of:

1. Identifying clonal rearrangements of the *IGH* chain gene
2. Assessing the extent of somatic hypermutation in the variable region of the immunoglobulin heavy chain gene
3. Evaluating new research and methods in malignancy studies

Summary and Explanation of the Test

These assays amplify either genomic DNA or complementary DNA (cDNA) that lies between the upstream leader (V_HL) or framework 1 (FR1) regions and the downstream joining (J_H) region of the *IGH* gene. The assays employ two different master mixes: Hypermutation Mix 1 and Hypermutation Mix 2. The Hypermutation Mix 1 targets sequences between the leader (V_HL) and joining (J_H) regions. Therefore the amplicon product(s) span the entire variable (V_H) region, which contains all framework (FR) and complementarity-determining regions (CDR). The Hypermutation Mix 2 targets sequences between the framework 1 (FR1) and joining (J_H) regions. The resulting amplicons include a portion of the FR1 region to the downstream J_H region. The primers that target the V_HL and FR1 regions have been redesigned to include a universal sequencing tag at the 5' end. This design allows for bi-directional sequencing of clonal PCR products with just one sequencing-tag specific forward primer and one J_H reverse primer, thus ensuring a more reliable and complete coverage of clonal products. Positive and negative DNA, positive RNA, as well as an amplification control are included in the assay. Clonality is indicated if any one of the master mixes generates clonal products.

Background

Rearrangements of the antigen receptor genes occur during ontogeny in B and T lymphocytes. These gene rearrangements are unique in length and sequence for each cell. Therefore, polymerase chain reaction (PCR) assays can be used to identify lymphocyte populations derived from a single cell by detecting the unique V-J gene rearrangements present within these antigen receptor loci.¹ This PCR-based assay employs multiple consensus DNA primers that target conserved genetic regions within the immunoglobulin heavy chain (*IGH*) gene. This test is used to detect and sequence the majority of clonal *IGH* rearrangements from either genomic DNA (gDNA) or complementary DNA (cDNA). Clonal products can be detected using a variety of methods, including gel and capillary electrophoresis. The presence of *IGH* somatic hypermutation (SHM) is defined as greater or equal to 2% difference from the germline variable (V) gene sequence, whereas less than 2% difference is considered evidence of no somatic hypermutation.³

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

1. P Ghia et al., *Leukemia* 21: 1-3 (2007).
2. P Ghia et al., *Blood* 105: 1678-1685 (2005).
3. F Davi et al., *Leukemia* 22: 212-214 (2008).

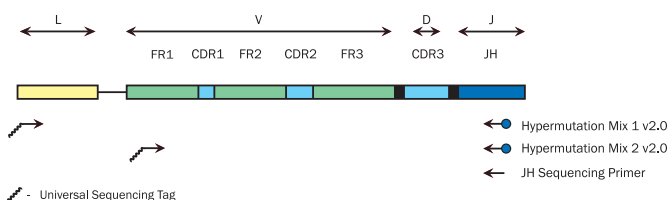


Figure Legend: Simple representation of the organization of a rearranged immunoglobulin heavy chain gene on chromosome 14. Black arrows represent the relative positions of primers that target the conserved Leader (L) and Framework 1 (FR1) regions, and the downstream consensus J_H gene segments.

Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0013 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0013 Clonal Control RNA	400 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
Master Mixes	Target	Units in Assay	Units in Assay MegaKit
Hypermutation Mix 1 v2.0	Leader + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
Hypermutation Mix 2 v2.0	Framework 1 + J _H	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes
Primers	Target	Units in Assay	Units in Assay MegaKit
Primer - Hypermutation	Leader + Framework 1	1 x 10 µL tube at 100 µM	5 x 10 µL tube
<i>IGH</i> J _H Primer	J _H	1 x 10 µL tube at 100 µM	5 x 10 µL tube

Ordering Information

Catalog #	Products	Quantity
5-101-0030	<i>IGH</i> Somatic Hypermutation Assay v2.0 - Gel Detection	33 reactions
5-101-0040	<i>IGH</i> Somatic Hypermutation Assay v2.0 MegaKit - Gel Detection	330 reactions
5-101-0031	<i>IGH</i> Somatic Hypermutation Assay v2.0 - ABI Fluorescence Detection	33 reactions
5-101-0041	<i>IGH</i> Somatic Hypermutation Assay v2.0 MegaKit - ABI Fluorescence Detection	330 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

FLT3 Mutation Assays

Assay Use

These Research Use Only assays identify *FLT3* mutations.

Summary and Explanation of the Test

FLT3 Mutation Assays target regions of the *FLT3* gene to identify internal tandem duplication (ITD) mutations and tyrosine kinase domain (TKD) mutations, such as the D835 and I836 mutations. DNA is amplified by PCR, TKD amplicon is enzymatically digested, and *FLT3* mutations are detected via agarose gel (catalog #14120010) or capillary (catalog #14120031) electrophoresis.

Assay kits include three PCR master mixes, along with positive and negative controls. *FLT3* ITD master mix tests for internal tandem duplication mutations. *FLT3* D835 master mix tests for TKD region mutations. The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

Master mixes contain fluorophore-labeled (capillary) or unlabeled (gel) primer sets as appropriate to kit detection method.

Background

FLT3 is a receptor tyrosine kinase that is normally expressed on many cell types including hematologic stem cells. Mutation of the *FLT3* receptor, by either internal tandem duplication (ITD) of the juxtamembrane domain or point mutation in the activation loop of the tyrosine kinase domain (TKD), causes constitutive activation of the *FLT3* receptor.

Such gain-of-function mutations in the FMS related tyrosine kinase 3 (*FLT3*) gene are the subject of research studies and multiple clinical trials targeting Acute Myeloid Leukemia (AML) subjects. The most prevalent type of *FLT3* mutation is an internal tandem duplication in and around the juxtamembrane domain. The second most common mutation type in the *FLT3* gene is a TKD point mutation in aspartate (D835) or isoleucine (I836).

Specimen Requirements

High quality genomic DNA

References

<https://clinicaltrials.gov>

1. Acute Myeloid Leukemia, Clinical Practice Guidelines in Oncology, National Comprehensive Cancer Network (v.2.2014).
2. Lowenberg, B. et al. "Acute myeloid leukemia." *N Engl J Med* 341(14):1051-62 (1999).
3. Thiede, C. et al. "Analysis of *FLT3*-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB and identification of subgroups with poor prognosis." *Blood* 99(12): 4326-35 (2002).
4. Nakao, M. et al. "Internal tandem duplication of the *FLT3* gene found in acute myeloid leukemia." *Leukemia* 10(12):1911-18 (1996).
5. Yamamoto, Y et al. Activating mutation of D835 within the activation loop of *FLT3* in human hematologic malignancies. *Blood*, 97(8):2434-9 (2001).
6. Gilliland, DG et al. The roles of *FLT3* in hematopoiesis and leukemia. *Blood* 100(5):1532-154 (2002).

These products are not available for sale or use in the United States.

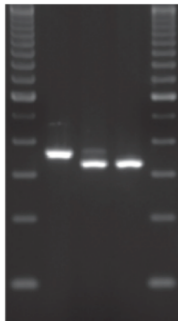
Reagents

Controls	Concentration	Units in 33 Reaction Assay
IVS-0017 Clonal Control DNA	200 µg/mL	1 x 100 µL tube
IVS-P001 Clonal Control DNA	200 µg/mL	1 x 100 µL tube
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube
Master Mixes	Target	Units in 33 Reaction Assay
<i>FLT3</i> ITD Master Mix	<i>FLT3</i> ITD	1 x 1500 µL tube
<i>FLT3</i> D835 Master Mix	<i>FLT3</i> TKD	1 x 1500 µL tube
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube

FLT3 ITD Master Mix

Lane 1 = 100% IVS-0050
Lane 2 = 10% IVS-0050
Lane 3 = 100% IVS-0000

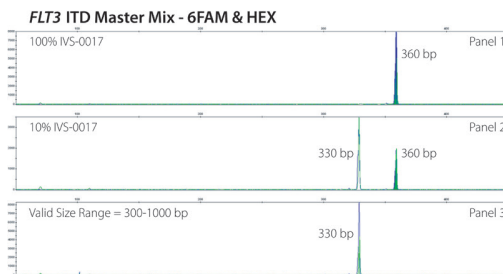
Mutant: 360 bp →
Wild Type: 330 bp →



Gel Electrophoresis Detection

Data was generated using the *FLT3* ITD Master Mix, and amplified products were run on a 2% TBE agarose gel alongside a 100bp DNA size ladder. Lane 1 is a *FLT3* ITD control*; lane 2 is a 10% dilution of a *FLT3* ITD control; and lane 3 is IVS-0000, which is representative of a wild type product.

*IVS-0050 performs comparable to IVS-0017 clonal control DNA, which is included in the kit as the positive control.



Capillary Electrophoresis Detection (ABI)

Data was generated using the *FLT3* ITD Master Mix and amplified products were run on an ABI 3100 instrument. Templates analyzed follow: Panel 1 is the *FLT3* ITD positive control; panel 2 is a 10% dilution of the positive control; and Panel 3 is IVS-0000, which is representative of a wild type product.

Ordering Information		
Catalog #	Products	Quantity
1-412-0010	<i>FLT3</i> Mutation Assay - Gel Detection	33 reactions
1-412-0031	<i>FLT3</i> Mutation Assay - ABI Fluorescence Detection	33 reactions

These products are for Research Use Only (RUO). Not intended for diagnostic purposes, and additionally not available for sale or use in regions where CE-IVD products are registered.

These products are not available for sale or use in the United States. Please see the LeukoStrat CDx *FLT3* Mutation Assay product pages for IVD product availability in the United States and other global regions.

Analyte Specific Reagents



invivoscribe.com/products/reagents/

Invivoscribe Analyte Specific Reagents (ASRs) target B- and T-cell antigen receptor loci, *FLT3* ITD and TKD, or chromosome translocations (*IGH* - *BCL2*, *BCR-ABL1*, *PML-RARA*).

Warranty and Liability

Invivoscribe, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe® shall have no liability for direct, indirect, consequential, or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

Notice

The products in this section are Analyte Specific Reagents. The analytical and performance characteristics are not established. Many of these products are covered by one or more patents including European Patent No. 1549764, European Patent No. 2418287, European Patent No. 2460889, Japanese Patent No. 4708029, United States Patent No. 8859748 and United States Patent No. 10280462. All of these patents are licensed to Invivoscribe®.

These products require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). No license under these patents to use amplification processes or enzymes is conveyed expressly or by implication to the purchaser by the purchase of these products.

Analyte Specific Reagents

Inivoscribe Analyte Specific Reagents (ASRs) target B- and T-cell antigen receptor loci, *FLT3* ITD and TKD, or chromosome translocations (*IGH* - *BCL2*, *BCR*-*ABL1*, *PML-RARA*).

The ASRs are available as a single tube containing a volume of 1500 μ L. To ensure the highest quality and reliability of reagents ASRs are manufactured under cGMP and ISO 13485 standards.

Per the current US FDA regulations, ASRs may only be sold to *in vitro* diagnostic manufacturers, CLIA accredited high complexity laboratories, VHA regulated clinical laboratories, and laboratories not intending to use ASRs as a part of a diagnostic test.

Analytical and performance characteristics are not established. ASRs are not available for sale or use outside of the USA.

FLT3 (FMS-like Tyrosine Kinase 3)

Description	Catalog #
<i>FLT3</i> ITD Master Mix - Unlabeled	Please contact Inivoscribe
<i>FLT3</i> TKD Master Mix - Unlabeled	Please contact Inivoscribe
<i>FLT3</i> ITD Master Mix - 6FAM & HEX - ASR	A-412-0071
<i>FLT3</i> TKD Master Mix - 6FAM - ASR	A-412-0081

IGH (Immunoglobulin Heavy Chain Gene Locus)

Description	Catalog #
<i>IGH</i> Framework 1 - 6FAM	A-101-0061
<i>IGH</i> Framework 2 - Unlabeled	A-101-0070
<i>IGH</i> Framework 2 - 6FAM	A-101-0091
<i>IGH</i> Framework 3 - Unlabeled	A-101-0080
<i>IGH</i> Framework 3 - HEX	A-101-0081
<i>IGH</i> FR1 - 6FAM	A-101-0011
<i>IGH</i> FR2 - Unlabeled	A-101-0020
<i>IGH</i> FR2 - 6FAM	A-101-0101
<i>IGH</i> FR3 - Unlabeled	A-101-0030
<i>IGH</i> FR3 - HEX	A-101-0031
<i>IGH</i> DH1 - 6 - HEX	A-101-0041
<i>IGH</i> DH7 - 6FAM	A-101-0051

IGK (Immunoglobulin Kappa Light Chain Gene Locus)

Description	Catalog #
<i>IGK</i> V - J - Unlabeled	A-102-0010
<i>IGK</i> V - J - 6FAM	A-102-0011
<i>IGK</i> V - K _{de} - Unlabeled	A-102-0020
<i>IGK</i> V - K _{de} - 6FAM	A-102-0021

IGL (Immunoglobulin Lambda Light Chain Gene Locus)

Description	Catalog #
<i>IGL</i> V - J - 6FAM	A-103-0011

TRB (T-Cell Receptor Beta Chain Gene Locus)

Description	Catalog #
<i>TCRB</i> V - J1 + 2 - Unlabeled	A-205-0010
<i>TCRB</i> V - J1 + 2 - 6FAM & HEX	A-205-0011
<i>TCRB</i> V - J2 - Unlabeled	A-205-0020
<i>TCRB</i> V - J2 - 6FAM	A-205-0021
<i>TCRB</i> D - J1 + 2 - 6FAM & HEX	A-205-0031

TRG (T-Cell Receptor Gamma Chain Gene Locus)

Description	Catalog #
<i>TCRG</i> V(2-5,8-11) J 1 + 2+P - 6FAM	A-207-0091
<i>TCRG</i> V(1-8,9) J - 6FAM	A-207-0071
<i>TCRG</i> V(1-8) J - HEX	A-207-0021

IGH-BCL2 t(14;18)

Description	Catalog #
<i>BCL2</i> /J _H Mbr - Unlabeled	A-309-0050
<i>BCL2</i> /J _H 3'Mbr - Unlabeled	A-309-0060
<i>BCL2</i> /J _H mcr - Unlabeled	A-309-0070

Controls, Reagents, and Enzymes

DNA Controls

- 126 Quick Reference for DNA Controls
- 130 Cell Line DNA Controls
- 130 LymphoTrack Low Positive Controls
- 131 LymphoQuant Internal Controls

RNA Controls

- 132 Quick Reference for RNA Controls
- 133 Cell Line RNA Controls
- 134 *BCR/ABL1* RNA Dilution Sets

Control Panels

- 136 DNA and RNA Sensitivity Panels
- 137 *BCR/ABL1* Proficiency Panel

Master Mix Controls

- 138 Amplification Control Master Mix
- 138 Specimen Control Size Ladder

Reagents

- 138 ABI Detection Reagents

Enzymes

- 139 EagleTaq DNA Polymerase*
- 139 FalconTaq DNA Polymerase

Warranty and Liability

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Notice to Purchaser - EagleTaq DNA Polymerase ONLY

*This product is for sale and use in the European Economic Area only. It is not to be resold or transferred to another party. The purchaser of this product may use this product only for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Human and veterinary diagnostic uses under Roche patent claims require a separate license from Roche. All uses other than internal research and human and veterinary diagnostic uses under Roche patent claims require a separate license from Thermo Fisher Scientific. By using this product, you acknowledge your agreement to the above. Further information on purchasing licenses from Roche may be obtained by contacting the Licensing Department of Roche Molecular Systems, Inc., 4300 Hacienda Drive, Pleasanton, California 94588, USA or Roche Diagnostics GmbH, Sandhofer Strasse 116, 68305 Mannheim, Germany. Further information on purchasing licenses from Thermo Fisher Scientific may be obtained by contacting the Licensing Department of Thermo Fisher Scientific, 5791 Van Allen Way, Carlsbad, California 92008, USA.

Controls, Reagents, and Enzymes

Invivoscribe offers an extensive range of General Purpose Reagents (GPRs) and Research Use Only (RUO) nucleic acid controls.

Controls are available in several different formats: plasmid DNA, DNA extracted from tissue or cell lines, or RNA extracted from cell lines. These controls can be purchased in various dilutions or as complete dilution sets and panels for several purposes, such as to help with assay validation, sensitivity or proficiency testing, or troubleshooting.

The following pages will provide an overview of available controls, along with a number of tables and reference guides, to help you decide which Invivoscribe control(s) will be suitable for your application.

DNA Controls

Every laboratory needs suitable controls (positive and negative) for sensitivity and proficiency testing, as well as for troubleshooting. Since patient samples cannot serve as true controls (due to a lack of characterization and inter-sample variability), Invivoscribe offers a multitude of high quality, reliable DNA controls manufactured under cGMP conditions.

These controls can be used for most assays targeting B- and T-cell antigen receptor loci, *FLT3* ITD and TKD loci, or *IGH-BCL2*, *BCR-ABL1*, and *PML-RAR α* chromosome translocations.

Quick Reference for DNA Controls

The vast majority of our high-quality DNA controls, including sensitivity controls and panels, are supplied in aliquots of 100 μ L and are adjusted to a final concentration of 200 μ g/mL in 1/10 TE (1 mM Tris- HCl (pH 8.0), 0.1 mM EDTA).

Positive for	Immunoglobulin Rearrangements			Mutations			Translocations		T-Cell Receptor Gene Rearrangements		
	<i>IGH</i>	<i>IGK</i>	<i>IGL</i>	<i>IGHV</i> SHM	<i>FLT3</i> ITD	<i>FLT3</i> TKD	<i>IGH-CCND1</i> **	<i>IGH-BCL2</i>	<i>TRB</i>	<i>TRG</i>	<i>TRD</i>
IVS-0001								◆			
IVS-0004									◆	◆	
IVS-0007	◆	◆	◆					◆			
IVS-0008 ^c	○								◆	◆	
IVS-0009									◆	◆	
IVS-0010	◆	◆					◆				
IVS-0013	◆	◆									
IVS-0019	◆	◆									
IVS-0021		◆							◆	◆	◆
IVS-0024	◆	◆									
IVS-0029	◆	◆									
IVS-0030 [†]	◆	◆		◆							
IVS-0031	◆	◆									
LymphoQuant B-cell Internal Control	★	★									
LymphoQuant T-cell Internal Control									★	★	
LymphoTrack B-cell Low Positive Control	★	★									
LymphoTrack T-cell Low Positive Control									★	★	
<i>FLT3</i> ITD Positive Control					◆						
<i>FLT3</i> TKD Positive Control						◆					

[†]These controls can be used as SHM positive controls with $\geq 2\%$ mutational rates compared to the germline sequence.

Tissue DNA

Standard Concentrations

The vast majority of our high-quality DNA controls, including sensitivity controls and panels, are supplied in aliquots of 100 μ L and are adjusted to a final concentration of 200 μ g/mL in 1/10 TE (1 mM Tris- HCl (pH 8.0), 0.1 mM EDTA). This diluent provides sufficient buffering capacity and EDTA to protect the DNA without interfering with the Mg^{2+} concentrations required for robust amplification reactions.

IVS-0000 Polyclonal Control DNA

Tissue DNA controls are extracted from normal, disease-free tissue and are tested extensively to ensure quality and reproducibility of your test results. IVS-0000 Polyclonal Control DNA consists of genomic DNA isolated from the tissue of normal human tonsils. This control represents an excellent negative control for gene rearrangements, chromosome translocations, and mutation tests and is included in all of our PCR DNA-based assay kits. This DNA is supplied at a volume of 100 μ L and at a concentration of 200 μ g/mL.

Catalog #	Description
4-092-0010	IVS-0000 Polyclonal Control DNA*

*These controls are general purpose reagents (GPRs). All others are research use only (RUO).

Cell Line DNA

Reliable Positive Controls

Cell Line DNA controls are extracted from established cell lines grown under cell culture conditions recommended by the supplier. Our controls are tested extensively to ensure quality and reproducibility of your test results. Please note, these controls are for qualitative use only.

Note | n/c is used to indicate that the control has not been fully characterized; there may be additional rearrangements, translocations or mutations associated with the control.

Standard Concentrations

The majority of our high-quality DNA controls, including our sensitivity panels, are supplied in aliquots of 100 μ L and are adjusted to a final concentration of 200 μ g/mL in 1/10 TE (1 mM Tris-HCl (pH 8.0), 0.1 mM EDTA). This diluent provides sufficient buffering capacity and EDTA to protect the DNA controls without interfering with the Mg^{2+} concentrations required for robust amplification reactions. DNA dilutions are diluted volume to volume (v/v) in our negative control DNA, IVS-0000 Polyclonal Control DNA.

IVS-0001 Clonal Control DNA

IVS-0001 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: n/c
Chromosome Translocations: *IGH-BCL2* t(14;18) mcr
Mutations: n/c

Catalog #	Description
4-088-0010	100% IVS-0001 Clonal Control DNA*

IVS-0004 Clonal Control DNA

IVS-0004 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *TRB, TRG*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-0190	100% IVS-0004 Clonal Control DNA*
4-088-0210	20% IVS-0004 Clonal Control DNA
4-088-0220	10% IVS-0004 Clonal Control DNA
4-088-0230	5% IVS-0004 Clonal Control DNA

IVS-0007 Clonal Control DNA

IVS-0007 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK, IGL*
Chromosome Translocations: *IGH-BCL2* t(14;18) Mbr
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-0370	100% IVS-0007 Clonal Control DNA*
4-088-0390	20% IVS-0007 Clonal Control DNA
4-088-0400	10% IVS-0007 Clonal Control DNA
4-088-0410	5% IVS-0007 Clonal Control DNA
4-088-0420	1% IVS-0007 Clonal Control DNA

IVS-0008 Clonal Control DNA

IVS-0008 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH* D_H-J_H^t, *TRB, TRG*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-0430	100% IVS-0008 Clonal Control DNA*
4-088-0470	5% IVS-0008 Clonal Control DNA
4-088-0480	1% IVS-0008 Clonal Control DNA

IVS-0009 Clonal Control DNA

IVS-0009 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *TRB, TRG*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-0490	100% IVS-0009 Clonal Control DNA*
4-088-0500	30% IVS-0009 Clonal Control DNA
4-088-0510	20% IVS-0009 Clonal Control DNA
4-088-0520	10% IVS-0009 Clonal Control DNA
4-088-0530	5% IVS-0009 Clonal Control DNA
4-088-0540	1% IVS-0009 Clonal Control DNA

* These controls are general purpose reagents (GPRs). All others are research use only (RUO).

Cell Line DNA

IVS-0010 Clonal Control DNA

IVS-0010 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK, IGL*
Chromosome Translocations: *IGH-BCL1 t(11;14)*
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-0550	100% IVS-0010 Clonal Control DNA*
4-088-0560	30% IVS-0010 Clonal Control DNA
4-088-0580	10% IVS-0010 Clonal Control DNA
4-088-0590	5% IVS-0010 Clonal Control DNA

IVS-0013 Clonal Control DNA

IVS-0013 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK, IGL*
Chromosome Translocations: n/c
Mutations: n/c

Catalog #	Description
4-088-0730	100% IVS-0013 Clonal Control DNA*

IVS-0019 Clonal Control DNA

IVS-0019 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-1090	100% IVS-0019 Clonal Control DNA*
4-088-1100	30% IVS-0019 Clonal Control DNA
4-088-1120	10% IVS-0019 Clonal Control DNA
4-088-1130	5% IVS-0019 Clonal Control DNA
4-088-1140	1% IVS-0019 Clonal Control DNA

IVS-0021 Clonal Control DNA

IVS-0021 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *TRB, TRD, TRG*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-1210	100% IVS-0021 Clonal Control DNA*
4-088-1230	20% IVS-0021 Clonal Control DNA
4-088-1240	10% IVS-0021 Clonal Control DNA
4-088-1250	5% IVS-0021 Clonal Control DNA
4-088-1260	1% IVS-0021 Clonal Control DNA

IVS-0024 Clonal Control DNA

IVS-0024 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as a 5% ready-to-use dilution into a standard negative control as listed in the table below.

Catalog #	Description
4-088-1390	100% IVS-0024 Clonal Control DNA*
4-088-1430	5% IVS-0024 Clonal Control DNA

* These controls are general purpose reagents (GPRs). All others are research use only (RUO).

‡ This control does not contain a complete *IGH V_H-J_H* rearrangement and may only be suitable for *IGH D_H-J_H* rearrangements.

IVS-0029 Clonal Control DNA

IVS-0029 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK, IGL*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-1690	100% IVS-0029 Clonal Control DNA*
4-088-1730	5% IVS-0029 Clonal Control DNA

IGH SHM Positive Control DNA

IGH SHM Positive Control can be used as a positive control for:

Gene Rearrangements: *IGH*
Chromosome Translocations: n/c
Mutations: *IGH SHM*

Catalog #	Description
4-088-0008	IGH SHM Positive Control DNA*

IVS-0030 Clonal Control DNA

IVS-0030 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK*
Chromosome Translocations: *IGH-BCL2 t(14;18) Mbr*
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-1750	100% IVS-0030 Clonal Control DNA*
4-088-1760	30% IVS-0030 Clonal Control DNA
4-088-1770	20% IVS-0030 Clonal Control DNA
4-088-1780	10% IVS-0030 Clonal Control DNA
4-088-1790	5% IVS-0030 Clonal Control DNA
4-088-1800	1% IVS-0030 Clonal Control DNA

IVS-0031 Clonal Control DNA

IVS-0031 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK*
Chromosome Translocations: *IGH-BCL2 t(14;18) mcr*
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-088-1810	100% IVS-0031 Clonal Control DNA*
4-088-1840	10% IVS-0031 Clonal Control DNA
4-088-1860	1% IVS-0031 Clonal Control DNA

* These controls are general purpose reagents (GPRs). All others are research use only (RUO).



Low Positive Controls

Minimal Residual Disease (MRD) testing is a valuable tool that allows investigators to study and monitor multiple myeloma (MM), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML) and other hematologic diseases. Recent treatment advances have led to significantly increased clinical response and overall survival, but ultimately most subjects will relapse, driving the need for sensitive MRD monitoring. Sensitive and standardized testing such as NGS-based MRD may one day enable identification of those cases that will eventually relapse versus those who are potentially cured. In addition to the need for more sensitive tracking, it is clear that standardized methods are needed. Currently, MRD methods are highly subjective and recommendations are often based on consensus expert-shared knowledge and experience, not on a validated, objective method. Once specific rearrangements have been identified, LymphoTrack assays can be used with LymphoQuant and LymphoTrack Low Positive Controls to track these clonotype populations to a sensitivity as low as 10^{-4} .

LymphoTrack® B-cell Low Positive Control

LymphoTrack B-cell Low Positive Control can be used as a control for:

Gene Rearrangements: *IGH*
Chromosome Translocations: n/c
Mutations: n/c

Catalog #	Description
4-088-0098	LymphoTrack® B-cell Low Positive Control*

LymphoTrack® T-cell Low Positive Control

LymphoTrack T-cell Low Positive Control can be used as a control for:

Gene Rearrangements: *TRB, TRG*
Chromosome Translocations: n/c
Mutations: n/c

Catalog #	Description
4-088-0108	LymphoTrack® T-cell Low Positive Control*

Note | Same product listed on page 60 in MRD Solution section.

*LymphoTrack® Low Positive Controls are research use only (RUO), not for diagnostic procedures.

LymphoQuant®

Internal Controls

LymphoQuant T-cell or B-cell Internal Controls may be spiked into specimens to estimate the respective number of clonotype T-cell or *IGH* equivalents present. Addition of the LymphoQuant Internal Control to the specimen PCR facilitates clonotype tracking over time without any additional sequencing cost. Consistent use of a LymphoQuant Internal Control enables investigators to objectively monitor the disease over time with a highly standardized, sensitive method. The LymphoTrack MRD software will help researchers that use the LymphoQuant Internal Control, calculate and report an estimated number of clonotype cell equivalents and the percent clonotype in the sample, enabling researchers and pharmaceutical companies to accurately monitor hematologic disease in longitudinal studies.

LymphoQuant® B-cell Internal Control

LymphoQuant B-cell Internal Control can be used to objectively track Ig clonotypes.

Gene Rearrangements: *IGH*
Chromosome Translocations: n/c
Mutations: n/c

Catalog #	Description
4-088-0118	LymphoQuant® B-cell Internal Control*

LymphoQuant® T-cell Internal Control

LymphoQuant T-cell Internal Control can be used to objectively track TCR clonotypes.

Gene Rearrangements: *TRB, TRG*
Chromosome Translocations: n/c
Mutations: n/c

Catalog #	Description
4-088-0128	LymphoQuant® T-cell Internal Control*

Note | Same product listed on page 61 in MRD Solution section.

*LymphoQuant® Internal Controls are research use only (RUO), not for diagnostic procedures.

RNA Controls

Quick Reference for RNA Controls

Reliable Assay Controls

Our RNA controls are extracted from well characterized cell lines grown under standard and carefully controlled culture conditions. The general purpose reagent (GPR) controls are tested to ensure linearity and reproducible results. Since this RNA is extracted from cell lines, these controls can be used with any of the standard housekeeping genes.

Standard Concentrations

Each RNA single control tube (as separate control tube, RNA sensitivity panel and proficiency panel) is supplied in aliquots of 100 μL at a final concentration of 400 $\mu\text{g}/\text{mL}$ in water. Each *BCR/ABL* RNA dilution set member is supplied in aliquots of 50 μL at a final concentration of 400 $\mu\text{g}/\text{mL}$ in water. To ensure maximum stability, the dilution set should be stored at $-85\text{ }^{\circ}\text{C}$ to $-65\text{ }^{\circ}\text{C}$ and the number of freeze-thaw cycles should be kept to a minimum.

RNAs positive for chromosome translocations			
Chromosome Translocation	Clonal Control RNA	Chromosome Translocation	Clonal Control RNA
<i>BCR-ABL1</i> t(9;22) p210 e13a2 (b2a2)	IVS-0003	<i>CBFB-MYH11</i> inv(16)	IVS-0015
<i>BCR-ABL1</i> t(9;22) p210 e14a2 (b3a2)	IVS-0011	<i>E2A-PBX1</i> t(1;19)(q23;p13)	IVS-0002
<i>BCR-ABL1</i> t(9;22) p190 e1a2	IVS-0032	<i>PML-RARA</i> t(15;17)(q22;q11)	IVS-0020

RNAs negative for chromosome translocations
IVS-0035 Clonal Control RNA is negative for <i>BCR-ABL</i> t(9;22) and <i>PML-RARa</i> t(15;17) chromosome translocations.
IVS-0035 may be used as a negative control for other chromosome translocations or diluents for other chromosome translocation positive controls. Please do not hesitate to contact us at sales@invivoscribe.com so we can evaluate whether this control may work for your testing needs.

These controls are research use only (RUO), not for diagnostic procedures.

Cell Line RNA

Reliable Positive and Negative Controls

Cell Line RNA controls are extracted from established cell lines grown under cell culture conditions recommended by the supplier. Our GPR controls are tested extensively to ensure quality and reproducibility of your test results. Please note, these controls are for qualitative use only.

Standard Concentrations

Our GMP-manufactured high-quality RNA controls, including sensitivity controls and proficiency panel samples, are supplied in aliquots of 100 μ L and are adjusted to a final concentration of 400 μ g/mL in RNase-free glass-distilled water. The pH of distilled water is slightly acidic; this protects the RNA from hydrolysis. RNA dilutions are diluted volume to volume in our negative control RNA, IVS-0035 Clonal Control RNA.

IVS-0002 Clonal Control RNA

IVS-0002 Clonal Control RNA can be used as a positive control for the chromosome translocation: *E2A-PBX1* t(1;19) (q23;p13).

Catalog #	Description
4-089-0100	IVS-0002 Clonal Control RNA*

IVS-0003 Clonal Control RNA

IVS-0003 Clonal Control RNA can be used as a positive control for the chromosome translocation: *BCR-ABL1* t(9;22) p210 e13a2 (b2a2).

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-089-0190	IVS-0003 Clonal Control RNA*
4-089-0200	10 ⁻¹ IVS-0003 Clonal Control RNA
4-089-0210	10 ⁻² IVS-0003 Clonal Control RNA
4-089-0220	10 ⁻³ IVS-0003 Clonal Control RNA
4-089-0230	10 ⁻⁴ IVS-0003 Clonal Control RNA
4-089-0240	10 ⁻⁵ IVS-0003 Clonal Control RNA
4-089-0250	10 ⁻⁶ IVS-0003 Clonal Control RNA

IVS-0011 Clonal Control RNA

IVS-0011 Clonal Control RNA can be used as a positive control for the chromosome translocation: *BCR-ABL1* t(9;22) p210 e14a2 (b3a2).

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-089-0910	IVS-0011 Clonal Control RNA*
4-089-0920	10 ⁻¹ IVS-0011 Clonal Control RNA
4-089-0930	10 ⁻² IVS-0011 Clonal Control RNA
4-089-0940	10 ⁻³ IVS-0011 Clonal Control RNA
4-089-0950	10 ⁻⁴ IVS-0011 Clonal Control RNA
4-089-0960	10 ⁻⁵ IVS-0011 Clonal Control RNA

IVS-0015 Clonal Control RNA

IVS-0015 Clonal Control RNA can be used as a positive control for the chromosome translocation: *CBFB-MYH11* inv(16)

Catalog #	Description
4-089-1270	IVS-0015 Clonal Control RNA*

IVS-0020 Clonal Control RNA

IVS-0020 Clonal Control RNA can be used as a positive control for the chromosome translocation: *PML-RARA* t(15;17) L-Form.

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-089-1720	IVS-0020 Clonal Control RNA*
4-089-1730	10 ⁻¹ IVS-0020 Clonal Control RNA
4-089-1740	10 ⁻² IVS-0020 Clonal Control RNA
4-089-1750	10 ⁻³ IVS-0020 Clonal Control RNA
4-089-1760	10 ⁻⁴ IVS-0020 Clonal Control RNA

IVS-0032 Clonal Control RNA

IVS-0032 Clonal Control RNA can be used as a positive control for the chromosome translocation: *BCR-ABL1* t(9;22) p190 e1a2.

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

Catalog #	Description
4-089-2800	IVS-0032 Clonal Control RNA*
4-089-2810	10 ⁻¹ IVS-0032 Clonal Control RNA
4-089-2820	10 ⁻² IVS-0032 Clonal Control RNA
4-089-2830	10 ⁻³ IVS-0032 Clonal Control RNA
4-089-2840	10 ⁻⁴ IVS-0032 Clonal Control RNA
4-089-2850	10 ⁻⁵ IVS-0032 Clonal Control RNA
4-089-2860	10 ⁻⁶ IVS-0032 Clonal Control RNA

IVS-0035 Clonal Control RNA

IVS-0035 Clonal Control RNA can be used as a negative control for *BCR-ABL1* t(9;22) and *PML-RARa* t(15;17) chromosome translocations.

Catalog #	Description
4-089-3070	IVS-0035 Clonal Control RNA*

* These controls are general purpose reagents (GPRs). All others are research use only (RUO).

BCR/ABL RNA Dilution Sets

Our *BCR/ABL* b2a2, b3a2, and e1a2 RNA Dilution Sets consist of RNA that has been extracted from *BCR-ABL1* expressing and *BCR-ABL1* negative cell lines. Each set is composed of several dilutions (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5}) of the *BCR-ABL1* positive RNA diluted (v/v) into RNA purified from a cell line that does not contain a *BCR-ABL1* translocation. Also included in these sets is a 100% *BCR-ABL1* negative RNA.

The individual *BCR/ABL* b2a2, b3a2, and e1a2 RNA Dilution Sets can be used as reference and validation materials with assays that target the main transcripts of *BCR-ABL1* t(9;22) translocations: p210 (e13a2 (b2a2), e14a2 (b3a2), and p190 (e1a2). These products may be used as the following:

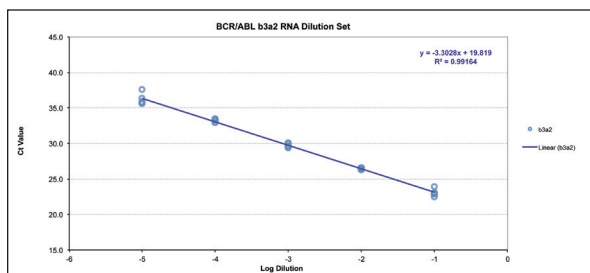
1. Routine testing controls for cDNA synthesis, amplification and detection
2. Controls to establish a standard reference curve
3. Proficiency controls
4. Sensitivity controls for specific target assays

Data

Plot of Ct values (5 replicates) for the 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} dilutions.

Ordering Information - e14a2 (b3a2)

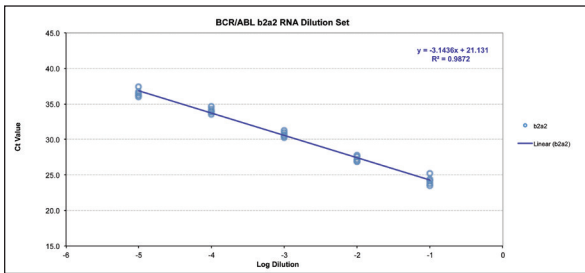
Catalog #	Description
4-085-0210	<i>BCR/ABL</i> b3a2 RNA Dilution Set (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} dilutions and negative)



These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

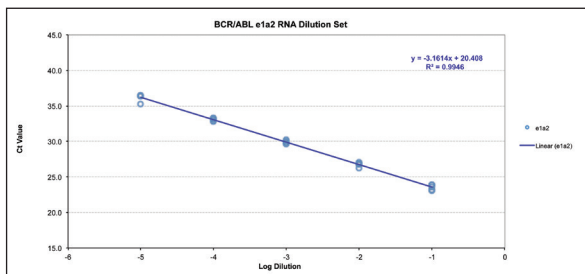
Ordering Information - e13a2 (b2a2)

Catalog #	Description
4-085-0310	BCR/ABL b2a2 RNA Dilution Set (10 ⁻¹ , 10 ⁻² , 10 ⁻³ , 10 ⁻⁴ , 10 ⁻⁵ dilutions and negative)



Ordering Information - e1a2

Catalog #	Description
4-085-0110	BCR/ABL e1a2 RNA Dilution Set (10 ⁻¹ , 10 ⁻² , 10 ⁻³ , 10 ⁻⁴ , 10 ⁻⁵ dilutions and negative)



These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

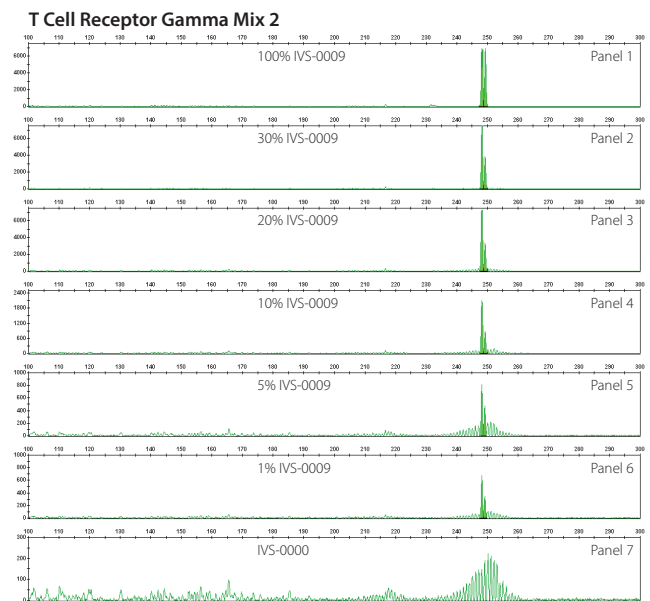
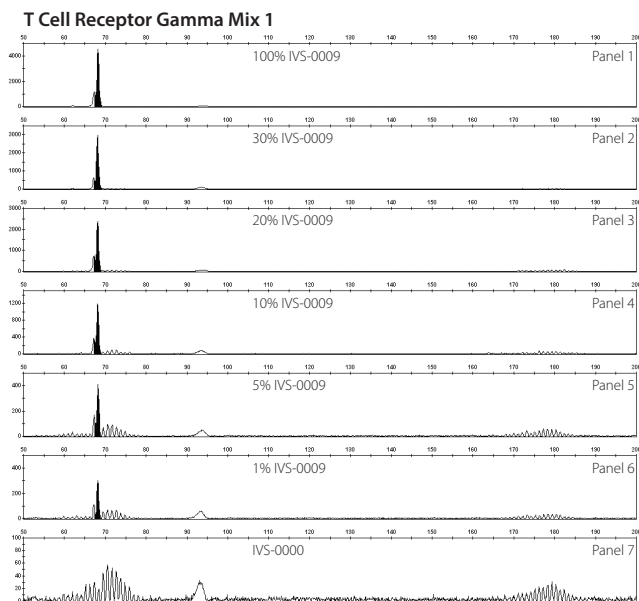
Control Panels

DNA Sensitivity Panels

DNA sensitivity panels are 6 member panels that consist of 100% clonal DNA extracted from a positive control cell line and 30%, 20%, 10%, 5%, and 1% dilutions of the positive clonal DNA diluted (v/v) into our standard negative control DNA, IVS-0000 Polyclonal Control DNA. Each tube contains 100 μ L of DNA at a concentration of 200 μ g/mL in 1/10 TE buffer (1 mM Tris-HCl pH 8.0, 0.1 mM EDTA). This diluent provides sufficient buffering capacity and EDTA to protect the DNA controls without interfering with the Mg^{2+} concentrations required for robust amplification reactions. Please note, these controls are for qualitative use only.

RNA Sensitivity Panels

RNA sensitivity panels are 7 member panels that consist of 100% clonal RNA extracted from a positive control cell line and 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , and 10^{-6} (1:10 - 1:1 000 000) dilutions of the positive clonal RNA diluted (v/v) into our standard negative control RNA, IVS-0035 Clonal Control RNA. Each tube contains 100 μ L of RNA at 400 μ g/mL in RNase-free glass-distilled water. The pH of distilled water is slightly acidic thereby protecting the RNA from hydrolysis. Please note, these controls are for qualitative use only.



This data was generated testing a Sensitivity Panel for IVS-0009 Clonal Control DNA using the master mixes listed. PCR products were run on an ABI 3130xL capillary electrophoresis instrument for differential fluorescence detection and data analyses. Panel 7 shows the polyclonal Gaussian distributions expected from our negative control IVS-0000 Polyclonal Control DNA. Data in the other panels are tests of positive control samples at the dilutions indicated. Clonal peaks (highlighted) are clearly evident in all of the positive sample panels.

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

DNA Sensitivity Panels

Catalog #	Description	Can be used as a positive control for:
4-086-0040	Sensitivity Panel for IVS-0004 Clonal Control DNA	<i>TRB, TRG</i>
4-086-0070	Sensitivity Panel for IVS-0007 Clonal Control DNA	<i>IGH, IGK, IGL, IGH-BCL2 t(14;18) Mbr</i>
4-086-0090	Sensitivity Panel for IVS-0009 Clonal Control DNA	<i>TRB, TRG</i>
4-086-0100	Sensitivity Panel for IVS-0010 Clonal Control DNA	<i>IGH, IGK, IGL, IGH-CCND1 t(11;14)</i>
4-086-0190	Sensitivity Panel for IVS-0019 Clonal Control DNA	<i>IGH, IGK</i>
4-086-0210	Sensitivity Panel for IVS-0021 Clonal Control DNA	<i>TRB, TRD, TRG</i>
4-086-0300	Sensitivity Panel for IVS-0030 Clonal Control DNA	<i>IGH, IGK, IGH-BCL2 t(14;18) Mbr</i>

RNA Sensitivity Panels

Catalog #	Description	Can be used as a positive control for:
4-087-0030	Sensitivity Panel for IVS-0003 Clonal Control RNA	<i>BCR-ABL1 t(9;22) p210 e13a2 (b2a2)</i>
4-087-0110	Sensitivity Panel for IVS-0011 Clonal Control RNA	<i>BCR-ABL1 t(9;22) p210 e14a2 (b3a2)</i>
4-087-0150	Sensitivity Panel for IVS-0015 Clonal Control RNA	<i>CBFB/MYH11 inv16</i>
4-087-0200	Sensitivity Panel for IVS-0020 Clonal Control RNA	<i>PML-RARA t(15;17) L-form</i>
4-087-0320	Sensitivity Panel for IVS-0032 Clonal Control RNA	<i>BCR-ABL1 t(9;22) p190 e1a2</i>

Proficiency Panel for *BCR-ABL1 t(9;22)*

This 10 member panel consists of 100% clonal control RNA extracted from three *BCR-ABL1* positive cell lines as well as 10^{-2} (1:100) and 10^{-4} (1:10,000) dilutions (v/v) of these positive RNAs diluted into a normal (*BCR-ABL1* negative) control RNA, IVS-0035 Clonal Control RNA. A sample of 100% IVS-0035 Clonal Control RNA is also included. All three cell lines (IVS-0003, IVS-0011, and IVS-0032) carry a t(9;22) translocation. One of the cell lines, IVS-0032, encodes for p190-type (ALL-associated) transcript with e1a2 junctions. The other two cell lines both encode for p210-type (CML-associated) transcripts. One of the p210-type translocations, IVS-0003, harbors a e13a2 (b2a2) junction and the other, IVS-0011, harbors a e14a2 (b3a2) junction. This proficiency panel is used to validate tests that identify *BCR-ABL1 t(9;22)* translocations.

RNA Proficiency Panel

Qty	Description	Chromosome Translocation
1	IVS-0003 Clonal Control RNA 10^{-2} IVS-0003 Clonal Control RNA 10^{-4} IVS-0003 Clonal Control RNA	<i>BCR-ABL1</i> p210 e13a2 (b2a2)
1	IVS-0011 Clonal Control RNA 10^{-2} IVS-0011 Clonal Control RNA 10^{-4} IVS-0011 Clonal Control RNA	<i>BCR-ABL1</i> p210 e14a2 (b3a2)
1	IVS-0032 Clonal Control RNA 10^{-2} IVS-0032 Clonal Control RNA 10^{-4} IVS-0032 Clonal Control RNA	<i>BCR-ABL1</i> p190 e1a2
1	IVS-0035 Clonal Control RNA	<i>BCR-ABL1</i> Negative

Ordering Information

Catalog #	Description
4-310-0100	Proficiency Panel for <i>BCR/ABL t(9;22)</i> translocations

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

Master Mix Controls

These master mixes serve as control for many of our DNA assays to ensure that sample DNA is of sufficient quality and integrity to generate a valid result.

Amplification Control Master Mix

Our Amplification Control master mix targets the *HLA-DQa* locus and generates a product of 235 base pair in size from human genomic DNA. This control is available in unlabeled (for Gel Detection) and fluorescence labeled format (for ABI Fluorescence Detection, 6FAM).

Catalog #	Description
2-096-0010	Amplification Control Master Mix - Unlabeled*
2-096-0011	Amplification Control Master Mix - 6FAM*

Specimen Control Size Ladder

Our Specimen Control Size Ladder master mix targets four different housekeeping genes producing products of approximately 100, 200, 300, 400, and 600 base pair in size to ensure that the quality and quantity of the sample DNA is adequate to yield a valid result with the specific assay(s).

This master mix is based on the BIOMED-2 Concerted Action BMH4-CT98-3936 from the EuroClonality Group and is available for Gel Detection (unlabeled) or ABI detection (labeled with 6FAM).

Catalog #	Description
2-096-0020	Specimen Control Size Ladder - Unlabeled*
2-096-0021	Specimen Control Size Ladder - 6FAM*

* These master mixes are general purpose reagents (GPRs).

Reagents

ABI Detection Reagents

Invivoscribe also offers highly deionized (Hi-Di) Formamide with ROX size standards for ABI fluorescence detection with the ABI 310 or 3100 series. Hi-Di Formamide is used to stabilize single strands of denatured PCR amplicons. The ROX size standards are fluorescent labeled DNA standards which cover the 50 to 400 base pair size range. Sizes of the individual standards are: 50, 60, 90, 100, 120, 150, 160, 180, 190, 200, 220, 240, 260, 280, 290, 300, 320, 340, 360, 380, and 400 base pair.

For samples tested on an ABI 310 or 3100 series, we recommend using 10 μ L of the Hi-Deionized Formamide with ROX Size Standards mixture for each microliter of PCR product. Please note that the ABI 310 and 3100 series require different concentrations of ROX size standards and the different Hi-Deionized Formamide with ROX Size Standards cannot be used interchangeably.

For samples tested on an ABI 3500 series, GeneScan™ 600® LIZ dye Size Standard v2.0 can be purchased from Thermo Fisher Scientific.

Ordering Information

Catalog #	Description
6-098-0051	Hi-Deionized Formamide with ROX Size Standard (ABI 310), 1 mL
6-098-0061	Hi-Deionized Formamide with ROX Size Standard (ABI 3100), 1 mL
Available through Thermo Fisher Scientific®: 4408399	GeneScan™ 600 LIZ® dye v2.0 Standard (ABI 3500), 800 reactions

For Research Use Only (RUO). Not intended for diagnostic purposes.

Enzymes

FalconTaq DNA Polymerase

FalconTaq DNA Polymerase can be used for amplification using PCR to obtain high specificity, sensitivity, and yield. This enzyme has been proven to minimize extension of non-specifically bound primers. Generate reliable results by using FalconTaq DNA Polymerase for robust performance.

Ordering Information

Catalog #	Description
6-097-0130	FalconTaq DNA Polymerase 250 U, 5 U/μL

For Research Use Only (RUO). Not intended for diagnostic purposes. The formulation and release criteria of FalconTaq DNA Polymerase are equivalent to Invivoscribe product number 60970100.

EagleTaq DNA Polymerase

Note | This product is for sale and use in the European Economic Area only. It is not to be resold or transferred to another party.

EagleTaq DNA Polymerase can be used to obtain highly specific and sensitive PCR amplification products. This enzyme has been proven to minimize extension of non-specifically bound primers. Obtain reliable results by using the gold standard of hot start polymerases for robust performance.

Ordering Information

Catalog #	Description
6-097-0100	EagleTaq DNA Polymerase 1000 U, 5 U/μL

For Research Use Only (RUO). Not intended for diagnostic purposes.

Custom Products



invivoscribe.com/clinical-lab-services/

The Invivoscribe team of experts can help develop your ideas into customized products. Allow us to partner with you to take a basic concept through design, development, validation, regulatory approval (if applicable), and release.

Warranty and Liability

Invivoscribe, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe shall have no liability for direct, indirect, consequential, or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

Custom Products

Customized Products to Meet Your Needs

The Invivoscribe team of experts can help develop your ideas into customized products. Allow us to partner with you to take a basic concept through design, development, validation, regulatory approval (if applicable), and release. For more information, please call our San Diego office at +1 858.224.6600 or send an email to sales@invivoscribe.com.

Custom Designed Assays

In response to the FDA announcing its intention to dramatically expand its regulatory oversight of laboratory developed tests (LDTs), Invivoscribe is partnering with laboratories worldwide to help facilitate the conversion of LDTs into FDA-cleared assays, as we know the barriers to bringing new assays online are often the availability of resources and the cost of validation.

By leveraging the power of our regulatory expertise, provided through each milestone, we can help ensure safety, efficacy and quality. Our customizable reagent manufacturing capabilities can reduce your LDT costs and lead to higher-quality testing.

To date, we have partnered with more than 40 laboratories around the world to develop, validate, and launch a variety of molecular products. A number of these partnerships have also led to the release of US and CE-marked *in vitro* diagnostic products and services. Learn how Invivoscribe can help you develop assays for new products, services, and novel applications.

Custom Controls and Validation Panels

We offer a large selection of well-characterized DNA and RNA controls that are used to define the performance characteristics of a wide variety of molecular reagents. To address your specific requirements, we can partner with you to design, validate, and provide custom controls and validation panels. If necessary, we are willing to acquire, characterize, and engineer custom controls for your specific application. We can produce DNA, RNA, or cDNA at any specified concentration, dilution, or volume. Please contact us with your requirements and we will be happy to provide controls to suit your needs.

Invivoscribe is a Comprehensive Partner for Companion Diagnostic Development

From biomarker identification through commercialization, Invivoscribe has expertise at every stage of companion diagnostics development.

- **Discovery & Patient Stratification:** We offer comprehensive gene panels to identify biomarkers and define patient populations, thus reducing development costs and improving the success of clinical trials.
- **Clinical Trials:** Our network of global laboratories accelerates sample acquisition and harmonizes testing to ensure accurate results.
- **Regulatory Approval:** Our in-house experts have experience seeking approval with global agencies.
- **Commercialization:** Our cGMP manufacturing expertise and distribution channels allow approved CDx to reach all global markets.

Invivoscribe is an ISO 13485-accredited and FDA/CDRH registered medical device manufacturer with a long record of successful partnerships. We are the industry-leading assay and software development company, providing full QSR design control and a complete range of cGMP manufactured assays, controls, reagents, and services to CLIA-accredited clinical laboratory and pharmaceutical communities.

Please contact us at +1 858.224.6600 or sales@invivoscribe.com for more details about partnering with Invivoscribe for the development and manufacturing of companion diagnostics, *in vitro* diagnostics, molecular reagents, and/or nucleic acid controls.

B- or T-cell Clonality Testing Workflow



invivoscribe.com/products/controls

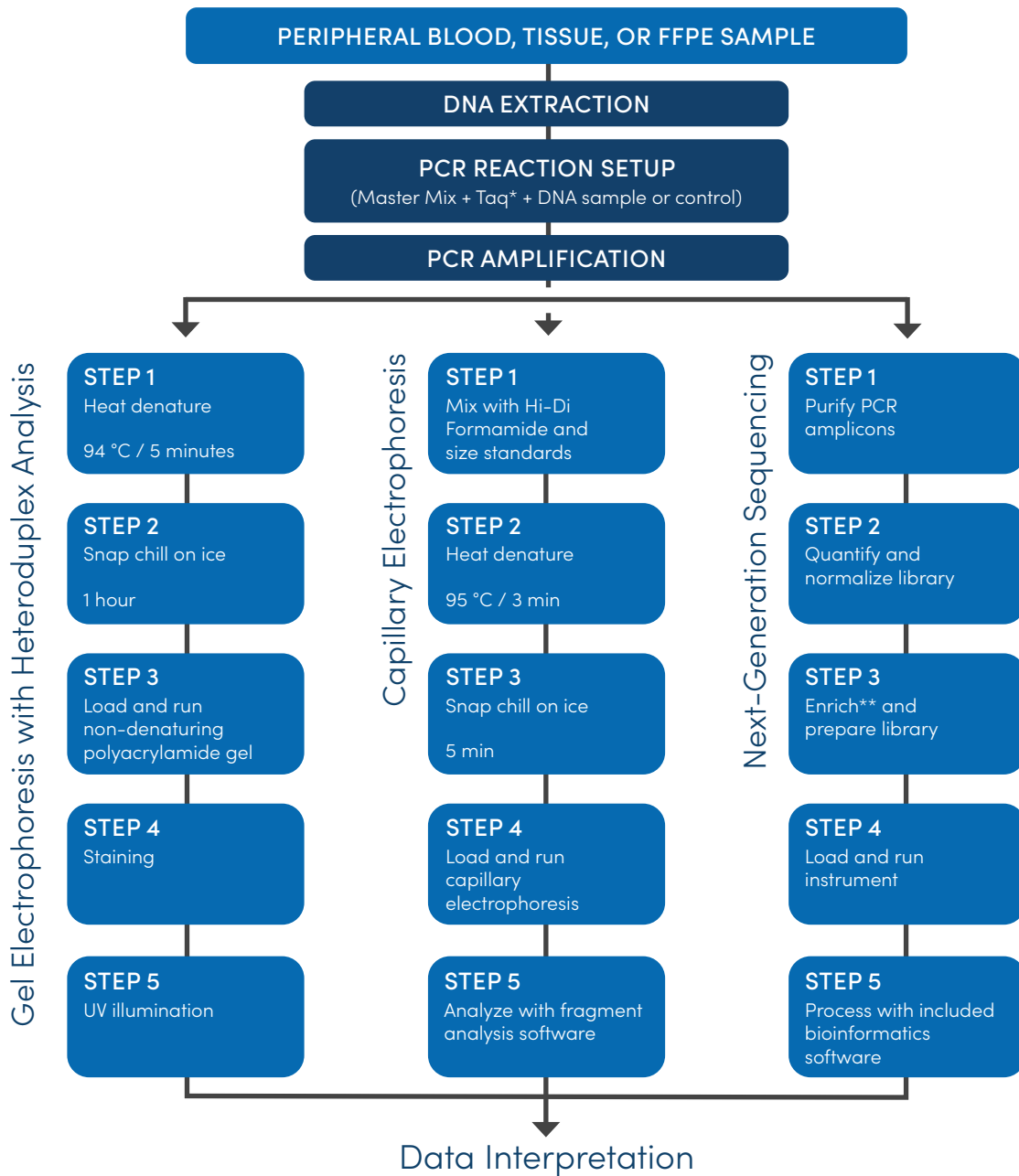
The Invivoscribe European Conformity marked *in vitro* diagnostics (CE-IVD) and Research Use Only (RUO) clonality assays detect clonal populations in just a few easy steps.

Warranty and Liability

Invivoscribe, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe® shall have no liability for direct, indirect, consequential, or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

B- or T-Cell Clonality Testing Workflow

The Invivoscribe European Conformity marked *in vitro* diagnostics (CE-IVD) and Research Use Only (RUO) clonality assays detect clonal populations in just a few easy steps. These steps include PCR amplification of the immunoglobulin or T-cell receptor genes of interest, followed by detection with non-denaturing polyacrylamide gels, capillary electrophoresis, or next-generation sequencing using an Illumina® MiSeq™, Thermo Fisher Scientific® Ion S5™ or PGM™ instrument. A flowchart illustrating this workflow is shown below.



*Or equivalent DNA Polymerase.

**For LymphoTrack and LymphoTrack Dx Assays run on the Ion S5 and PGM only.

CE-marked *in vitro* diagnostic products are not available for sale or use within North America.

LymphoTrack Dx and LymphoTrack Workflow Summary

illumina® MiSeq™



STEP 1 Prepare DNA samples

STEP 2 Run PCR with a LymphoTrack Assay

STEP 3 Purify amplicons

STEP 4 Quantify and prepare library

STEP 5 Load onto MiSeq™ flow cell and run

STEP 6 Process raw data with our included bioinformatics software

STEP 7 Analyze results

Notice | The LymphoTrack Dx Assays are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.
*Image courtesy of Illumina, Inc.

LymphoTrack Dx and LymphoTrack Workflow Summary

Thermo Fisher Scientific® Ion S5/PGM™



STEP 1 Prepare DNA samples

STEP 2 Run PCR with a LymphoTrack Assay

STEP 3 Purify amplicons

STEP 4 Quantify, prepare, and enrich library

STEP 5 Load onto Ion S5/PGM™ chip and run

STEP 6 Process raw data with our included bioinformatics software

STEP 7 Analyze results

Notice | The LymphoTrack Dx Assays are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.
*Image courtesy of Thermo Fisher Scientific

Next-Generation Sequencing Menu

Invivoscribe offers LymphoTrack and LymphoTrack Dx Assays for the analysis of B- and T-cell clonality, somatic hypermutation, and minimal residual disease studies**. Assays are designed for use on both industry standard next-generation sequencing (NGS) platforms: the Illumina® MiSeq™ and Thermo Fisher Scientific® Ion PGM™ and Ion S5™ instruments.

Invivoscribe assays for the Illumina® MiSeq™ platform offer the ability to analyze up to twenty two samples and two controls per gene target and the multiplexing capabilities to generate a sequencing library that combines amplicons from different Invivoscribe LymphoTrack and LymphoTrack Dx Assays onto the same flow cell. Our included software then sorts and assigns the correct sequences to their corresponding sample.

Invivoscribe assays for the Ion PGM™ and Ion S5™ platform offer the ability to analyze up to ten samples and two controls per gene target and the multiplexing capability to generate a sequencing library that combines amplicons from different Invivoscribe LymphoTrack and LymphoTrack Dx Assays onto the same sequencing chip, reducing per sample testing costs.

All LymphoTrack and LymphoTrack Dx Assays allow for fast and easy analysis and data visualization using the included bioinformatics software. The LymphoTrack software sorts and assigns the sequences to their corresponding sample and provides information such as the prevalence, gene segment usage, and the mutation rate (*IGH* Leader and *IGH* FR1 only). In addition, the Invivoscribe Minimal Residual Disease (MRD) Software allows for clonotype sequences to be tracked in subsequent samples for research applications.

The table below indicates which LymphoTrack (Research Use Only) and LymphoTrack Dx (CE-IVD Marked) Assays are currently available.

CE-Marked IVD Assays	MiSeq™	Ion S5/PGM™
LymphoTrack® Dx <i>IGHV</i> Leader Somatic Hypermutation Assays	AVAILABLE	NOT AVAILABLE
LymphoTrack® Dx <i>IGH</i> FR1 Assays	AVAILABLE	AVAILABLE
LymphoTrack® Dx <i>IGH</i> FR2 Assays	AVAILABLE	AVAILABLE
LymphoTrack® Dx <i>IGH</i> FR3 Assays	AVAILABLE	AVAILABLE
LymphoTrack® Dx <i>IGH</i> FR1/2/3 Assays	AVAILABLE	AVAILABLE
LymphoTrack® Dx <i>IGK</i> Assays	AVAILABLE	AVAILABLE
LymphoTrack® Dx <i>TRG</i> Assays	AVAILABLE	AVAILABLE
LymphoTrack® Dx <i>TRB</i> Assays	AVAILABLE	NOT AVAILABLE
Research Use Only (RUO) Assays	MiSeq™	Ion S5/PGM™
LymphoTrack® <i>IGHV</i> Somatic Hypermutation Assays	AVAILABLE	PLEASE INQUIRE
LymphoTrack® <i>IGH</i> FR1 Assays	AVAILABLE	AVAILABLE
LymphoTrack® <i>IGH</i> FR2 Assays	AVAILABLE	AVAILABLE
LymphoTrack® <i>IGH</i> FR3 Assays	AVAILABLE	AVAILABLE
LymphoTrack® <i>IGH</i> FR1/2/3 Assays	AVAILABLE	AVAILABLE
LymphoTrack® <i>IGK</i> Assays	AVAILABLE	AVAILABLE
LymphoTrack® <i>TRG</i> Assays	AVAILABLE	AVAILABLE
LymphoTrack® <i>TRB</i> Assay	AVAILABLE	NOT AVAILABLE

CE-marked assays are *in vitro* diagnostic products and are not available for sale or use within North America.

**Minimal residual disease (MRD) applications are currently for research use only.

Gel and Capillary Electrophoresis Menu

Inivoscribe offers assays that can be analyzed using two conventional methods of fragment analysis: gel electrophoresis or capillary electrophoresis. Gel electrophoresis kits offer a comparatively easy and inexpensive solution for clonality, translocation, and mutational testing and are often the method of choice for laboratories new to using these methods and techniques. PCR products are analyzed using non-denaturing polyacrylamide gels (PAGE) and often require a heteroduplex step for resolution of generated amplicons.

Capillary electrophoresis kits are supplied with fluorescently labeled primers, allowing the resulting PCR products to be analyzed on Applied Biosystems (ABI) platforms e.g. 3130, 3500, 3500xL, 3500xL Dx. Fragment analysis by capillary electrophoresis offers the ability to detect fragments with a high level of accuracy and analytical sensitivity and allows for greater sample throughput compared to gel detection methods. In addition, capillary electrophoresis detection often facilitates a more objective interpretation of results than gel-based detection.

The table below summarizes which detection methods are available for our clonality, translocation and *FLT3* mutation assays either as RUO, CE-IVD, or IVD.

CE-Marked IVD Assays	Gel	ABI
IdentiClone® <i>IGH + IGK</i> B-Cell Clonality Assay	NOT AVAILABLE	AVAILABLE
IdentiClone® <i>IGH</i> Gene Clonality Assay	NOT AVAILABLE	AVAILABLE
IdentiClone® <i>IGK</i> Gene Clonality Assay	NOT AVAILABLE	AVAILABLE
IdentiClone® <i>IGL</i> Gene Clonality Assay	NOT AVAILABLE	AVAILABLE
IdentiClone® <i>TCRB</i> Gene Clonality Assay	NOT AVAILABLE	AVAILABLE
IdentiClone® T-Cell Receptor Gamma Gene Rearrangement Assay 2.0	NOT AVAILABLE	AVAILABLE
IdentiClone® <i>TCRD</i> Gene Clonality Assay	NOT AVAILABLE	AVAILABLE
LeukoStrat® <i>FLT3</i> Mutation Assay 2.0	NOT AVAILABLE	AVAILABLE
LeukoStrat® CDx <i>FLT3</i> Mutation Assay (CE-marked)	NOT AVAILABLE	AVAILABLE
IVD Companion Diagnostic Assays	Gel	ABI
LeukoStrat® CDx <i>FLT3</i> Mutation Assay IVD (USA)	NOT AVAILABLE	AVAILABLE
LeukoStrat® CDx <i>FLT3</i> Mutation Assay (JP)	NOT AVAILABLE	AVAILABLE
LeukoStrat® CDx <i>FLT3</i> Mutation Assay (CE-IVD)	NOT AVAILABLE	AVAILABLE
Research Use Only (RUO) Assays	Gel	ABI
<i>FLT3</i> Mutation Assay	AVAILABLE	AVAILABLE
<i>IGH + IGK</i> B-Cell Clonality Assay	AVAILABLE	AVAILABLE
<i>IGH</i> Gene Rearrangement Assay	AVAILABLE	AVAILABLE
<i>IGH</i> Gene Clonality Assay	AVAILABLE	AVAILABLE
<i>IGH</i> Somatic Hypermutation Assay v2.0	AVAILABLE	AVAILABLE
<i>IGL</i> Gene Clonality Assay	AVAILABLE	AVAILABLE
<i>TCRB</i> Gene Clonality Assay	AVAILABLE	AVAILABLE
T-Cell Receptor Gamma Gene Rearrangement Assay	AVAILABLE	AVAILABLE
T-Cell Receptor Gamma Gene Rearrangement Assay 2.0	AVAILABLE	AVAILABLE
<i>TCRD</i> Gene Clonality Assay	AVAILABLE	AVAILABLE
<i>BCL1/JH</i> Translocation Assay	AVAILABLE	NOT AVAILABLE
<i>BCL2/JH</i> Translocation Assay	AVAILABLE	NOT AVAILABLE
<i>BCL2/JH</i> t(14;18) Translocation Assay	AVAILABLE	NOT AVAILABLE
BCR/ABL t(9;22) Translocation Assay	AVAILABLE	AVAILABLE
PML/RARα t(15;17) Translocation Assay	AVAILABLE	AVAILABLE

CE-marked assays are *in vitro* diagnostic products and are available in regions that accept CE-IVD products. IVD assays are *in vitro* diagnostic products, and are available for sale or use in regions indicated.

Common Technical Support Questions

1. What sample types may be suitable for analysis with Invivoscribe Gel and Capillary assays?

We recommend high-quality DNA for clonality testing with our assays. This can be extracted from frozen or fresh tissue, peripheral blood, bone marrow, skin biopsies, etc.

2. When should the recommended controls be run with our assays?

The no template, positive, and negative controls should be included in every run for each target, per the product insert or instructions for use.

3. What is the purpose of the Specimen Control Size Ladder and Amplification Control master mix? What is the difference between these master mixes?

The Specimen Control Size Ladder and Amplification Control master mixes are used as troubleshooting tools that allow you to determine if the quality and quantity of your DNA sample is suitable for use with our assays. The Specimen Control Size Ladder amplifies DNA at approximately 100, 200, 300, 400, and 600 base pairs; whereas, the Amplification Control amplifies DNA at 235 bp.

4. How should the master mix and controls be stored and thawed?

The master mixes should be stored at -65 to -85 °C and should be thawed at room temperature and vortexed prior to use. If you intend to use master mixes multiple times, we recommend aliquoting the master mixes to minimize the number of freeze/thaw cycles. For the *FLT3* CDx Mutation Assay: Opened vials of master mixes stored frozen may incur up to 4 freeze thaw cycles. Opened vials of controls stored frozen may incur up to 8 freeze thaw cycles.

5. Where can more information about the primers used in our assays be found?

Most primer information is proprietary to Invivoscribe and cannot be disclosed. We can, however, tell you the target area for the primers in each master mix, if you contact our support team by emailing support@invivoscribe.com or by calling +1 858-224-6600.

6. Which targets are recommended for the study of B-cell malignancies?

The EuroClonality/BIOMED-2 Group has shown that combined testing of *IGH* and *IGK* achieves a clinical sensitivity of 99%. If purchasing these assays separately is cost prohibitive, our *IGH* + *IGK* Gene Clonality Assay (does not include *IGH* Tubes D and E) may be a feasible alternative option (see Figure 2 and Table 1 in *Leukemia* (2007) 21, 201-206). We also offer next-generation sequencing LymphoTrack® Assays for *IGH* and *IGK* for use with MiSeq™ or Ion S5/PGM™ instruments. In addition, a high percentage of B-ALL patients have *TRG* rearrangements, which can be detected using our assays to detect *TRG* gene rearrangements.

7. What are the differences between our *IGH* Gene Rearrangement Assays and the *IGH* Gene Clonality Assays?

The *IGH* Gene Rearrangement Assay was designed by Invivoscribe; whereas, the *IGH* Gene Clonality Assay was designed by the EuroClonality/BIOMED-2 Group. Both assays target the conserved *IGH* framework regions, Framework 1, Framework 2, and Framework 3. The *IGH* Gene Clonality Assay also targets incomplete $D_H - J_H$ rearrangements. The *IGH* Gene Clonality Assay includes 33 reactions per master mix and the *IGH* Gene Rearrangement Assay includes 30 reactions per master mix.

8. What do *IGH* Tubes D and E target do and why are they challenging to interpret?

Tubes D and E of our *IGH* Gene Clonality Assays target incomplete *IGH* $D_H - J_H$ rearrangements. It is common to see known amplicons listed in the instructions for use in cases where a polyclonal background is absent (this is likely because these rearrangements are rare). Some of our customers are concerned by this, especially because there may be some samples that have robust germline amplification greater than the valid size range. We do not expect the germline amplification to outcompete true $D_H - J_H$ rearrangements. PCR amplicons generated from germline templates are much larger than true $D_H - J_H$ rearrangements. As a result, PCR products of germline amplifications are less robust when a specific target is present in samples.

9. Why does the polyclonal control produce a peak around 148 bp when amplified with *IGK* Tube A – 6FAM?

The 148 bp peak is a result of the restricted repertoire of *IGK* and this peak commonly appears flanked by several smaller peaks on each side. It is still possible to have a true clonal rearrangement at this size in samples. If you suspect that this peak is clonal in one of your samples, we recommend following up with heteroduplex analysis. Alternatively, NGS-based LymphoTrack® and LymphoTrack® Dx Assays provide an easier interpretation for *IGK* and reduces the number of master mixes to just one reaction.

10. What T-cell receptor kits would you recommend to detect T-cell clonal rearrangements?

Ideally, you should perform tests for *TRB*, *TRG*, and *TRD* to achieve the highest sensitivity. The EuroClonality/BIOMED-2 Group has shown that testing both *TRB* and *TRG* offers roughly the same sensitivity for the detection of T-cell malignancies as testing all three targets; however, they highly recommend testing all three assays in parallel to achieve optimal clinical sensitivity. *TRD* is especially useful in cases of suspected immature T-cell proliferations (see Figure 2 and Table 2 in *Leukemia* (2007) 21, 201-206). We also offer NGS kits for *TRG* for use with MiSeq™ or Ion S5/PGM™ instruments and for *TRB* for use with MiSeq™.

11. What are the differences between the *TCRG* Gene Clonality Assay and the T-Cell Receptor Gamma Gene Rearrangement Assay 2.0?

The *TCRG* Gene Clonality Assay was designed by the EuroClonality/BIOMED-2 Group and consists of two master mixes. For polyclonal populations, four Gaussian distributions are generated. The T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 was designed by Invivoscribe and its performance was subsequently reviewed and validated by the EuroClonality/BIOMED-2 Group. It targets all functional $V_H - J_H$ rearrangements in a single master mix and produces smaller amplicons grouped under a single Gaussian distribution. This allows for easier interpretation and makes the assay more suitable for DNA extracted from FFPE tissue, which may consist of partially degraded DNA that would not amplify well with the larger valid size range of the *TCRG* Gene Clonality Assay.

12. What are the differences between the *IGH-BCL2* Translocation Assay and the *IGH-BCL2* t(14;18) Translocation Assay?

The *IGH-BCL2* Translocation Assay was designed by the EuroClonality/BIOMED-2 Group and is available as either a CE-IVD or research use only assay whereas; the *IGH-BCL2* t(14;18) Translocation Assay was designed by Invivoscribe and is only available for research use only. Both of these assays target MBR and Mcr translocations, but the *IGH-BCL2* Translocation

Assay also targets the translocations at the 3' Mbr. The *IGH-BCL2* t(14;18) Translocation Assay was designed as a nested PCR allowing greater sensitivities (1 clonal cell per 10,000 normal cells) to be achieved. The limit of detection of the *IGH-BCL2* Translocation Assay is 1 clonal cell per 100 normal cells. Lastly, the *IGH-BCL2* Translocation Assay includes 33 reactions, whereas the *IGH-BCL2* t(14;18) Translocation Assay includes 30 reactions.

13. Do you offer quantitative chromosome translocation (e.g., BCR-ABL1) controls?

Our controls are validated for qualitative use, although our customers do successfully use them with quantitative assays. Unfortunately, we cannot guarantee their performance with any assay that was not designed by Invivoscribe.

14. Which capillary electrophoresis instruments are currently validated for use with our assay kits?

Currently the capillary electrophoresis instruments Invivoscribe has validated include: ABI 3100 and 3130 series for all capillary electrophoresis detection assays. The ABI 310 and 3500 instrument series have also been validated for the majority of our capillary electrophoresis detection assays. We are not able to support using instruments not listed as validated in the instructions for use of our CE-IVD assays.

15. What are the recommended settings for my ABI instrument?

Instruments should be calibrated with the DS-30 matrix standards (Dye set D) for the ABI 310, 3100, or 3130 instrument series. For the ABI 3500 sequencer series, we advise that you calibrate the instrument with DS-33 matrix standards. We also recommend using either POP-4 or POP-7 depending on which ABI instrument you are using. If your equipment supports POP-7, we recommend using this polymer as it can be utilized for both fragment analysis and sequencing; whereas, POP-4 can only be utilized for fragment analysis.

16. How should peaks outside the valid size range be interpreted when using assay kits?

You should not interpret peaks outside of the valid size range; although, in theory, it is possible to have a true rearrangement fall outside this region. If you are concerned about a suspect peak, you may sequence your product for confirmation. Please note that samples should always be interpreted within the context of all available clinical information.

17. Is cell-free DNA (cfDNA) a suitable sample type for Invivoscribe LymphoTrack® or LymphoTrack® Dx Assays?

The average size of cfDNA (~170 bps) makes it a suitable sample type to run with *IGH* FR3 master mixes. The use of cfDNA with *TRG* master mixes might be possible, but expected amplicon sizes generated with this assay are near the upper limits of the fragment lengths typically found with this sample type.

18. Is DNA extracted from FFPE tissue suitable to use with Invivoscribe LymphoTrack® or LymphoTrack® Dx Assays?

To ensure DNA from challenging specimens is of sufficient quality and quantity to generate a valid result, samples may be tested with the Specimen Control Size Ladder master mix.

19. On which instruments can I use the LymphoTrack® and LymphoTrack® Dx Assays?

We have different versions of our assays for the S5/PGM™ and MiSeq™ instruments (LymphoTrack *TRB* is currently available only on MiSeq™). No other DNA sequencers (e.g. 454) are currently

supported. Assays for the Ion S5/PGM™ and MiSeq™ platforms differ slightly in terms of the total number of indices, etc., but both have similar benefits such as a one-step PCR reaction and included bioinformatics software.

20. How much DNA is needed for the LymphoTrack® and LymphoTrack® Dx Assays?

50 ng of high-quality genomic DNA is required for the Ion S5/PGM™ and MiSeq™ LymphoTrack and LymphoTrack Dx Assays for clonality and somatic hypermutation applications.

21. Can I use a different library quantification method or kit?

We recommend using the KAPA™ kit for MiSeq™ assays and either the 2100 Bioanalyzer® or the LabChip® GX for the Ion S5/PGM™ assays.

22. Will the LymphoTrack® or LymphoTrack® Dx analysis software work on my computer?

The software requires Microsoft Windows 7 and Windows 10 (64-bit) and Excel 2007, 2010, or 2013 and will work with most desktop or laptop PCs. For specific requirements please refer to the software instructions for use.

23. Can I use the LymphoTrack® or LymphoTrack® Dx bioinformatics software with a different assay?

No, the software will only work with datasets obtained by our LymphoTrack and LymphoTrack Dx Assays.

24. What characters can I use when naming my samples and the file pathways? What types of files are accepted by the LymphoTrack® and LymphoTrack® Dx Software - MiSeq™?

Our software only recognizes file names and pathways that contain the following characters (A-Z, a-z, 0-9, . (dot), _ (underscore), - (hyphen)). In addition, spaces in the pathname for the data files or software (pathnames include file folders and file names) should be avoided. If the software encounters a character that is not listed above or extra spaces, an error message may be generated. Furthermore, the software is only compatible with adaptor-trimmed fastq.gz files that are generated by the MiSeq™ Reporter Software when the MiSeq™ instrument is used. An example of the naming format that the MiSeq™ Reporter uses: SampleName_S1_L001_R1_001.fastq.gz and SampleName_S1_L001_R2_001.fastq.gz.

25. Do the Invivoscribe MiSeq™ indices correspond to the Illumina® indices?

The indices included in our MiSeq™ master mixes follow Illumina®'s TruSeq LT nomenclature. For instance, *IGH* FR1 MiSeq™ 01 corresponds to A001. Information for the other indices can be found in the instructions for use on how to set up the MiSeq™ Sample Sheet to detect the appropriate indices.

26. Why am I getting a low percent passing filter and Q30 score?

Low Q30 and percent passing filter (%PF) scores could be an indication that the flow cell is overloaded. If this is suspected, verify your amplicon and library calculations and quantifications are correct. Low run metrics can also be attributed to many additional factors including poor quality DNA, contamination, flow cell or instrument issues, etc. Please refer to your Illumina MiSeq™ user guides and contact Illumina® Support.

27. Why is the same V_H-J_H rearrangement combination and sequence shared by two groups of reads, one of which is several bases shorter than the other when looking at the Read Summary tab of the excel document created by the LymphoTrack® Visualization Tool?

Our software was designed to list every unique sequence separately in order for the customer to see all of the data and make their own determination on how to interpret it. The several base pair difference can be due to a number of factors including amplification errors and sequencing errors. It could also be a result of similarities between some of the primer sequences that were designed to ensure maximum coverage. We also include a Merged Read Summary report for your reference that combines sequences that only differ by 1 or 2 basepairs.

28. Do I need to perform an adapter ligation prior to sequencing my products?

Performing an adapter ligation is not needed. The primers included in our LymphoTrack and LymphoTrack Dx master mixes already include the appropriate index barcodes and adapter sequences. After PCR amplification, you will be able to proceed with amplicon purification, amplicon quantification, library pooling, and sequencing.

29. If the LymphoTrack® or LymphoTrack® Dx software generated an error, what information should I submit to Technical Support?

Please submit the *.txt Log file that should have been created by the software in the output folder, a screenshot of the sample directory, and the Lot Number of the software CD you are using to support@invivoscribe.com.

30. Are controls provided with the kits? Can you purchase additional controls? How are they supplied?

Each kit contains the necessary positive and negative controls required to perform the assay; additional controls may also be purchased separately. Single-tube DNA controls are provided as 100 µL aliquots of 200 µg/mL in 1/10 TE Buffer, 50 µL aliquots of 50 µg/mL in 1/10 TE Buffer, and 45 µL aliquots of 15 µg/mL in 1/10 TE Buffer. Single-tube RNA controls are provided as 100 µL aliquots of 400 µg/ml in RNase free in glass distilled water.

31. What are the differences between dilution sets, sensitivity panels, and proficiency panels?

31a. RNA Dilution Sets

BCR/ABL b3a2 (Cat# 4-085-0210), BCR/ABL b2a2 (Cat# 4-085-0310), and BCR/ABL e1a2 (Cat# 4-085-0110). These sets contain six tubes: 100% negative control RNA and volume to volume (v/v) dilutions (10⁻¹, 10⁻², 10⁻³, 10⁻⁴, and 10⁻⁵) of the positive control RNA into the negative control RNA (IVS-0048). The RNA Dilution Sets are supplied at a concentration of 400 µg/mL, and each tube contains 50 µL. These dilution sets may be used to establish a standard reference curve, as proficiency controls, as sensitivity controls for specific target assays, and as routine testing controls for cDNA synthesis, amplification and detection.

31b. RNA Sensitivity Panels

These panels consist of seven tubes: 100% positive control RNA and v/v dilutions (10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, and 10⁻⁶) of the positive control RNA into the negative control RNA (IVS-0035). The RNA Sensitivity Panels are supplied at a

concentration of 400 µg/mL, and each tube contains 100 µL. The RNA Sensitivity Panels may be used as sensitivity controls for specific target assays, and as routine testing controls for cDNA synthesis, amplification and detection.

31c. DNA Sensitivity Panels

Consist of six tubes: 100% clonal DNA and v/v dilutions of the clonal DNA into negative polyclonal DNA (IVS-0000) to make 30%, 20%, 10%, 5%, and 1% dilutions. The DNA Sensitivity Panels are supplied at a concentration of 200 µg/mL and each tube contains 100 µL. The DNA Sensitivity Panels may be used as sensitivity controls for specific target assays.

31d. RNA Proficiency Panel

The proficiency panel for BCR-ABL1 t(9;22) can be used as a sensitivity control for specific target assays, and as routine testing controls for cDNA synthesis, amplification and detection. It consists of ten tubes: 100% positive control RNA and v/v dilutions (10⁻² and 10⁻⁴) of IVS-0003, IVS-0011 and IVS-0032. It also includes BCR-ABL1 Negative Clonal Control RNA (IVS-0035).

Product List by Catalog Number

Companion Diagnostic Assays

Capillary Analysis

K-412-0291	LeukoStrat® CDx <i>FLT3</i> Mutation Assay (CE-IVD)
K-412-0281	LeukoStrat® CDx <i>FLT3</i> Software (CE-IVD)
K-412-0331	LeukoStrat® CDx <i>FLT3</i> Mutation Assay (JP)
K-412-0341	LeukoStrat® CDx <i>FLT3</i> Software (JP)
K-412-0361	LeukoStrat® CDx <i>FLT3</i> Mutation Assay (USA)
K-412-0371	LeukoStrat® CDx <i>FLT3</i> Software (USA)

Research Use Only Assays

Capillary & Gel Fragment Analysis

1-100-0010	<i>IGH</i> + <i>IGK</i> B-Cell Clonality Assay – Gel Detection
1-100-0031	<i>IGH</i> + <i>IGK</i> B-Cell Clonality Assay – ABI Fluorescence Detection
1-100-0041	<i>IGH</i> + <i>IGK</i> B-Cell Clonality Assay MegaKit – ABI Fluorescence Detection
1-101-0010	<i>IGH</i> Gene Rearrangement Assay – Gel Detection
1-101-0020	<i>IGH</i> Gene Clonality Assay – Gel Detection
1-101-0040	<i>IGH</i> Gene Clonality Assay MegaKit – Gel Detection
1-101-0051	<i>IGH</i> Gene Rearrangement Assay – ABI Fluorescence Detection
1-101-0061	<i>IGH</i> Gene Clonality Assay – ABI Fluorescence Detection
1-101-0071	<i>IGH</i> Gene Rearrangement Assay MegaKit – ABI Fluorescence Detection
1-101-0081	<i>IGH</i> Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-102-0020	<i>IGK</i> Gene Clonality Assay – Gel Detection
1-102-0021	<i>IGK</i> Gene Clonality Assay – ABI Fluorescence Detection
1-102-0030	<i>IGK</i> Gene Clonality Assay MegaKit – Gel Detection
1-102-0031	<i>IGK</i> Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-103-0010	<i>IGL</i> Gene Clonality Assay – Gel Detection
1-103-0011	<i>IGL</i> Gene Clonality Assay – ABI Fluorescence Detection
1-103-0020	<i>IGL</i> Gene Clonality Assay MegaKit – Gel Detection
1-103-0021	<i>IGL</i> Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-205-0010	<i>TCRB</i> Gene Clonality Assay – Gel Detection
1-205-0011	<i>TCRB</i> Gene Clonality Assay – ABI Fluorescence Detection
1-205-0020	<i>TCRB</i> Gene Clonality Assay MegaKit – Gel Detection
1-205-0021	<i>TCRB</i> Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-206-0010	<i>TCRD</i> Gene Clonality Assay – Gel Detection
1-206-0011	<i>TCRD</i> Gene Clonality Assay – ABI Fluorescence Detection
1-206-0020	<i>TCRD</i> Gene Clonality Assay MegaKit – Gel Detection
1-206-0021	<i>TCRD</i> Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-207-0010	T-Cell Receptor Gamma Gene Rearrangement Assay – Gel Detection
1-207-0051	T-Cell Receptor Gamma Gene Rearrangement Assay – ABI Fluorescence Detection
1-207-0101	T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 – ABI Fluorescence Detection
1-207-0111	T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 MegaKit – ABI Fluorescence Detection
1-308-0010	<i>BCL1/IGH</i> Translocation Assay – Gel Detection
1-308-0020	<i>BCL1/IGH</i> Translocation Assay MegaKit – Gel Detection
1-309-0010	<i>BCL2/IGH</i> t(14;18) Translocation Assay – Gel Detection
1-309-0020	<i>BCL2/IGH</i> Translocation Assay – ABI Fluorescence Detection
1-309-0040	<i>BCL2/IGH</i> Translocation Assay MegaKit – Gel Detection
1-310-0010	<i>BCR/ABL</i> t(9;22) Translocation Assay – Gel Detection
1-310-0031	<i>BCR/ABL</i> t(9;22) Translocation Assay – ABI Fluorescence Detection
1-311-0010	<i>PML/RARα</i> t(15;17) Translocation Assay – Gel Detection
1-311-0011	<i>PML/RARα</i> t(15;17) Translocation Assay – ABI Fluorescence Detection
1-412-0010	<i>FLT3</i> Mutation Assay – Gel Detection
1-412-0031	<i>FLT3</i> Mutation Assay – ABI Fluorescence Detection

Master Mixes

2-096-0010	Amplification Control Master Mix – Unlabeled
2-096-0011	Amplification Control Master Mix – 6FAM
2-096-0020	Specimen Control Size Ladder – Unlabeled
2-096-0021	Specimen Control Size Ladder – 6FAM
2-101-0010	<i>IGH</i> Tube A – Unlabeled
2-101-0011	<i>IGH</i> Tube A – 6FAM
2-101-0020	<i>IGH</i> Tube B – Unlabeled
2-101-0030	<i>IGH</i> Tube C – Unlabeled
2-101-0031	<i>IGH</i> Tube C – HEX
2-101-0040	<i>IGH</i> Tube D – Unlabeled
2-101-0041	<i>IGH</i> Tube D – HEX
2-101-0050	<i>IGH</i> Tube E – Unlabeled
2-101-0051	<i>IGH</i> Tube E – 6FAM
2-101-0060	<i>IGH</i> Framework 1 (FR1) – Unlabeled
2-101-0061	<i>IGH</i> Framework 1 (FR1) – 6FAM
2-101-0070	<i>IGH</i> Framework 2 (FR2) – Unlabeled
2-101-0081	<i>IGH</i> Framework 3 (FR3) – HEX
2-101-0091	<i>IGH</i> Framework 2 (FR2) – 6FAM
2-101-0101	<i>IGH</i> Tube B – 6FAM
2-101-0170	Hypermutation Mix 1 v2.0 – Unlabeled
2-101-0171	Hypermutation Mix 1 v2.0 – 6FAM
2-101-0180	Hypermutation Mix 2 v2.0 – Unlabeled

2-101-0181	Hypermutation Mix 2 v2.0 – 6FAM
2-102-0010	<i>IGK</i> Tube A – Unlabeled
2-102-0011	<i>IGK</i> Tube A – 6FAM
2-102-0020	<i>IGK</i> Tube B – Unlabeled
2-102-0021	<i>IGK</i> Tube B – 6FAM
2-103-0010	<i>IGL</i> Tube – Unlabeled
2-103-0011	<i>IGL</i> Tube – 6FAM
2-205-0010	<i>TCRB</i> Tube A – Unlabeled
2-205-0011	<i>TCRB</i> Tube A – 6FAM & HEX
2-205-0020	<i>TCRB</i> Tube B – Unlabeled
2-205-0021	<i>TCRB</i> Tube B – 6FAM
2-205-0030	<i>TCRB</i> Tube C – Unlabeled
2-205-0031	<i>TCRB</i> Tube C – 6FAM & HEX
2-206-0010	<i>TCRD</i> Tube – Unlabeled
2-206-0011	<i>TCRD</i> Tube – 6FAM & HEX
2-207-0010	T-Cell Receptor Gamma Mix 1 – Unlabeled
2-207-0020	T-Cell Receptor Gamma Mix 2 – Unlabeled
2-207-0021	T-Cell Receptor Gamma Mix 2 – HEX
2-207-0071	T-Cell Receptor Gamma Mix 1 – 6FAM
2-207-0091	<i>TCRG</i> – 6FAM
2-308-0010	<i>BCL1/IGH</i> Tube – Unlabeled
2-309-0010	<i>BCL2/IGH</i> t(14;18) (Mbr) Mix 1b – Unlabeled
2-309-0020	<i>BCL2/IGH</i> t(14;18) (mcr) Mix 2b – Unlabeled
2-309-0030	<i>BCL2/IGH</i> t(14;18) (Mbr) Mix 1a – Unlabeled
2-309-0040	<i>BCL2/IGH</i> t(14;18) (mcr) Mix 2a – Unlabeled
2-309-0050	<i>BCL2/IGH</i> Tube A – Unlabeled
2-309-0060	<i>BCL2/IGH</i> Tube B – Unlabeled
2-309-0070	<i>BCL2/IGH</i> Tube C – Unlabeled
2-310-0010	<i>BCR/ABL</i> t(9;22) Mix 1a – Unlabeled
2-310-0020	<i>BCR/ABL</i> t(9;22) Mix 2a – Unlabeled
2-310-0030	<i>BCR/ABL</i> t(9;22) Mix 3a – Unlabeled
2-310-0040	<i>BCR/ABL</i> t(9;22) Mix 1b – Unlabeled
2-310-0041	<i>BCR/ABL</i> t(9;22) Mix 1b – HEX
2-310-0050	<i>BCR/ABL</i> t(9;22) Mix 2b – Unlabeled
2-310-0051	<i>BCR/ABL</i> t(9;22) Mix 2b – HEX
2-310-0060	<i>BCR/ABL</i> t(9;22) Mix 2c – Unlabeled
2-310-0061	<i>BCR/ABL</i> t(9;22) Mix 2c – HEX
2-310-0070	<i>BCR/ABL</i> t(9;22) Mix 3b – Unlabeled
2-310-0071	<i>BCR/ABL</i> t(9;22) Mix 3b – 6FAM
2-310-0080	<i>BCR/ABL</i> t(9;22) Mix 3c – Unlabeled
2-310-0081	<i>BCR/ABL</i> t(9;22) Mix 3c – 6FAM
2-310-0090	<i>BCR/ABL</i> t(9;22) Mix 3d – Unlabeled
2-310-0101	<i>BCR/ABL</i> t(9;22) Mix 3d – 6FAM
2-311-0011	<i>PML/RARα</i> t(15;17) Mix 1 – HEX
2-311-0031	<i>PML/RARα</i> t(15;17) Mix 2b – HEX
2-311-0041	<i>PML/RARα</i> t(15;17) Mix 2c – HEX

BCR/ABL RNA Dilution Sets

4-085-0110	<i>BCR/ABL</i> e1a2 RNA Dilution Set
4-085-0210	<i>BCR/ABL</i> b3a2 RNA Dilution Set
4-085-0310	<i>BCR/ABL</i> b2a2 RNA Dilution Set

Next-Generation Sequencing RUO LymphoTrack Assays

7-121-0059	LymphoTrack® <i>IGHV</i> Somatic Hypermutation Assay Kit A – MiSeq™
7-121-0069	LymphoTrack® <i>IGHV</i> Somatic Hypermutation Assay Panel – MiSeq™
7-121-0129	LymphoTrack® <i>IGH</i> FR1/2/3 Assay Kit A – MiSeq™
7-121-0139	LymphoTrack® <i>IGH</i> FR1/2/3 Assay Panel – MiSeq™
7-121-0009	LymphoTrack® <i>IGH</i> FR1 Assay Kit A – MiSeq™
7-121-0039	LymphoTrack® <i>IGH</i> FR1 Assay Panel – MiSeq™
7-121-0089	LymphoTrack® <i>IGH</i> FR2 Assay Kit A – MiSeq™
7-121-0099	LymphoTrack® <i>IGH</i> FR2 Assay Panel – MiSeq™
7-121-0109	LymphoTrack® <i>IGH</i> FR3 Assay Kit A – MiSeq™
7-121-0119	LymphoTrack® <i>IGH</i> FR3 Assay Panel – MiSeq™
7-121-0057	LymphoTrack® <i>IGH</i> FR1/2/3 Assay – S5/PGM™
7-121-0007	LymphoTrack® <i>IGH</i> FR1 Assay – S5/PGM™
7-121-0037	LymphoTrack® <i>IGH</i> FR2 Assay – S5/PGM™
7-121-0047	LymphoTrack® <i>IGH</i> FR3 Assay – S5/PGM™
7-122-0009	LymphoTrack® <i>IGK</i> Assay Kit A – MiSeq™
7-122-0019	LymphoTrack® <i>IGK</i> Assay Panel – MiSeq™
7-122-0007	LymphoTrack® <i>IGK</i> Assay – S5/PGM™
7-225-0009	LymphoTrack® <i>TRB</i> Assay Kit A – MiSeq™
7-225-0019	LymphoTrack® <i>TRB</i> Assay Panel – MiSeq™
7-227-0019	LymphoTrack® <i>TRG</i> Assay Kit A – MiSeq™
7-227-0009	LymphoTrack® <i>TRG</i> Assay Panel – MiSeq™
7-227-0007	LymphoTrack® <i>TRG</i> Assay – S5/PGM™
7-500-0007	LymphoTrack® Software – S5/PGM™
7-500-0008	LymphoTrack® MRD Software
7-500-0009	LymphoTrack® Software – MiSeq™

Capillary Fragment Analysis CE-IVD IdentiClone® Assays

9-100-0031	IdentiClone® IGH + IGK B-Cell Clonality Assay – ABI Fluorescence Detection
9-100-0041	IdentiClone® IGH + IGK B-Cell Clonality Assay MegaKit – ABI Fluorescence Detection
9-101-0061	IdentiClone® IGH Gene Clonality Assay – ABI Fluorescence Detection
9-101-0081	IdentiClone® IGH Gene Clonality Assay MegaKit – ABI Fluorescence Detection
9-102-0021	IdentiClone® IGK Gene Clonality Assay – ABI Fluorescence Detection
9-102-0031	IdentiClone® IGK Gene Clonality Assay MegaKit – ABI Fluorescence Detection
9-103-0011	IdentiClone® IGL Gene Clonality Assay – ABI Fluorescence Detection
9-103-0021	IdentiClone® IGL Gene Clonality Assay MegaKit – ABI Fluorescence Detection
9-205-0011	IdentiClone® TCRB Gene Clonality Assay – ABI Fluorescence Detection
9-205-0021	IdentiClone® TCRB Gene Clonality Assay MegaKit – ABI Fluorescence Detection
9-206-0011	IdentiClone® TCRD Gene Clonality Assay – ABI Fluorescence Detection
9-206-0021	IdentiClone® TCRD Gene Clonality Assay MegaKit – ABI Fluorescence Detection
9-207-0101	IdentiClone® T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 – ABI Fluorescence Detection
9-207-0111	IdentiClone® T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 MegaKit – ABI Fluorescence Detection

Capillary Fragment Analysis CE-IVD LeukoStrat® Assays

9-412-0091	LeukoStrat® FLT3 Mutation Assay 2.0 – ABI Fluorescence Detection
9-412-0101	LeukoStrat® FLT3 Mutation Assay 2.0 MegaKit – ABI Fluorescence Detection
K-412-0291	LeukoStrat® CDx FLT3 Mutation Assay 33 reactions – ABI Fluorescence Detection
K-412-0281	LeukoStrat® CDx FLT3 Mutation Assay Software

Analyte Specific Reagents

A-412-0071	FLT3 ITD MM – 6FAM & HEX – ASR
A-412-0081	FLT3 TKD MM – 6FAM – ASR
A-101-0011	IGH FR1 – 6FAM
A-101-0020	IGH FR2 – Unlabeled
A-101-0030	IGH FR3 – Unlabeled
A-101-0031	IGH FR3 – HEX
A-101-0041	IGH DH1 – 6 – HEX
A-101-0051	IGH DH7 – 6FAM
A-101-0061	IGH Framework 1 – 6FAM
A-101-0070	IGH Framework 2 – Unlabeled
A-101-0080	IGH Framework 3 – Unlabeled
A-101-0081	IGH Framework 3 – HEX
A-101-0091	IGH Framework 2 – 6FAM
A-101-0101	IGH FR2 – 6FAM
A-102-0010	IGKV – J – Unlabeled
A-102-0011	IGKV – J – 6FAM
A-102-0020	IGKV – K _{de} – Unlabeled
A-102-0021	IGKV – K _{de} – 6FAM
A-103-0011	IGLV – J – 6FAM
A-205-0010	TCRB V – J1+2 – Unlabeled
A-205-0011	TCRB V – J1+2 – 6FAM & HEX
A-205-0020	TCRB V – J2 – Unlabeled
A-205-0021	TCRB V – J2 – 6FAM
A-205-0031	TCRB D – J1+2 – 6FAM & HEX
A-207-0021	TCRG V(1-8)J – HEX
A-207-0071	TCRG V(1-8,9)J – 6FAM
A-207-0091	TCRG V(2-5,8-11)J1+2+P – 6FAM
A-309-0050	BCL2J _H Mbr – Unlabeled
A-309-0060	BCL2J _H 3'Mbr – Unlabeled
A-309-0070	BCL2J _H mcr – Unlabeled

BCR/ABL RNA Dilution Sets

4-085-0110	BCR/ABL e1a2 RNA Dilution Set
4-085-0210	BCR/ABL b3a2 RNA Dilution Set
4-085-0310	BCR/ABL b2a2 RNA Dilution Set

DNA Sensitivity Panels

4-086-0040	Sensitivity Panel – IVS-0004 Clonal Control DNA
4-086-0070	Sensitivity Panel – IVS-0007 Clonal Control DNA
4-086-0090	Sensitivity Panel – IVS-0009 Clonal Control DNA
4-086-0100	Sensitivity Panel – IVS-0010 Clonal Control DNA
4-086-0190	Sensitivity Panel – IVS-0019 Clonal Control DNA
4-086-0210	Sensitivity Panel – IVS-0021 Clonal Control DNA
4-086-0300	Sensitivity Panel – IVS-0030 Clonal Control DNA

RNA Sensitivity Panels

4-087-0030	Sensitivity Panel – IVS-0003 Clonal Control RNA
4-087-0110	Sensitivity Panel – IVS-0011 Clonal Control RNA
4-087-0150	Sensitivity Panel – IVS-0015 Clonal Control RNA
4-087-0200	Sensitivity Panel – IVS-0020 Clonal Control RNA
4-087-0320	Sensitivity Panel – IVS-0032 Clonal Control RNA

Cell Line DNA Controls

R-088-0230	FLT3 ITD Positive Control DNA – GPR
R-088-0240	FLT3 TKD Positive Control DNA – GPR
R-088-0250	FLT3 Extraction Control – GPR
4-088-0008	IGH SHM Positive Control DNA
4-088-0010	IVS-0001 Clonal Control DNA
4-088-0098	LymphoTrack® B-cell Low Positive Control
4-088-0108	LymphoTrack® T-cell Low Positive Control
4-088-0118	LymphoQuant® B-cell Internal Control
4-088-0128	LymphoQuant® T-cell Internal Control
4-088-0190	IVS-0004 Clonal Control DNA
4-088-0210	20% IVS-0004 Clonal Control DNA
4-088-0220	10% IVS-0004 Clonal Control DNA
4-088-0230	5% IVS-0004 Clonal Control DNA
4-088-0370	IVS-0007 Clonal Control DNA
4-088-0390	20% IVS-0007 Clonal Control DNA
4-088-0400	10% IVS-0007 Clonal Control DNA
4-088-0410	5% IVS-0007 Clonal Control DNA
4-088-0420	1% IVS-0007 Clonal Control DNA
4-088-0430	IVS-0008 Clonal Control DNA
4-088-0470	5% IVS-0008 Clonal Control DNA
4-088-0480	1% IVS-0008 Clonal Control DNA
4-088-0490	IVS-0009 Clonal Control DNA
4-088-0500	30% IVS-0009 Clonal Control DNA
4-088-0510	20% IVS-0009 Clonal Control DNA
4-088-0520	10% IVS-0009 Clonal Control DNA
4-088-0530	5% IVS-0009 Clonal Control DNA
4-088-0540	1% IVS-0009 Clonal Control DNA
4-088-0550	IVS-0010 Clonal Control DNA
4-088-0560	30% IVS-0010 Clonal Control DNA
4-088-0580	10% IVS-0010 Clonal Control DNA
4-088-0590	5% IVS-0010 Clonal Control DNA
4-088-0730	IVS-0013 Clonal Control DNA
4-088-1090	IVS-0019 Clonal Control DNA
4-088-1100	30% IVS-0019 Clonal Control DNA
4-088-1110	20% IVS-0019 Clonal Control DNA
4-088-1120	10% IVS-0019 Clonal Control DNA
4-088-1130	5% IVS-0019 Clonal Control DNA
4-088-1140	1% IVS-0019 Clonal Control DNA
4-088-1210	IVS-0021 Clonal Control DNA
4-088-1220	30% IVS-0021 Clonal Control DNA
4-088-1230	20% IVS-0021 Clonal Control DNA
4-088-1240	10% IVS-0021 Clonal Control DNA
4-088-1250	5% IVS-0021 Clonal Control DNA
4-088-1260	1% IVS-0021 Clonal Control DNA
4-088-1390	IVS-0024 Clonal Control DNA
4-088-1430	5% IVS-0024 Clonal Control DNA
4-088-1690	IVS-0029 Clonal Control DNA
4-088-1700	30% IVS-0029 Clonal Control DNA
4-088-1730	5% IVS-0029 Clonal Control DNA
4-088-1750	IVS-0030 Clonal Control DNA
4-088-1760	30% IVS-0030 Clonal Control DNA
4-088-1770	20% IVS-0030 Clonal Control DNA
4-088-1780	10% IVS-0030 Clonal Control DNA
4-088-1790	5% IVS-0030 Clonal Control DNA
4-088-1800	1% IVS-0030 Clonal Control DNA
4-088-1810	IVS-0031 Clonal Control DNA
4-088-1840	10% IVS-0031 Clonal Control DNA
4-088-1860	1% IVS-0031 Clonal Control DNA

Cell Line RNA Controls

4-089-0100	IVS-0002 Clonal Control RNA
4-089-0190	IVS-0003 Clonal Control RNA
4-089-0200	10 ⁻¹ IVS-0003 Clonal Control RNA
4-089-0210	10 ⁻² IVS-0003 Clonal Control RNA
4-089-0220	10 ⁻³ IVS-0003 Clonal Control RNA
4-089-0230	10 ⁻⁴ IVS-0003 Clonal Control RNA
4-089-0240	10 ⁻⁵ IVS-0003 Clonal Control RNA
4-089-0250	10 ⁻⁶ IVS-0003 Clonal Control RNA
4-089-0910	IVS-0011 Clonal Control RNA
4-089-0920	10 ⁻¹ IVS-0011 Clonal Control RNA
4-089-0930	10 ⁻² IVS-0011 Clonal Control RNA
4-089-0940	10 ⁻³ IVS-0011 Clonal Control RNA
4-089-0950	10 ⁻⁴ IVS-0011 Clonal Control RNA
4-089-0960	10 ⁻⁵ IVS-0011 Clonal Control RNA
4-089-1270	IVS-0015 Clonal Control RNA
4-089-1720	IVS-0020 Clonal Control RNA
4-089-1730	10 ⁻¹ IVS-0020 Clonal Control RNA
4-089-1740	10 ⁻² IVS-0020 Clonal Control RNA
4-089-1750	10 ⁻³ IVS-0020 Clonal Control RNA
4-089-1760	10 ⁻⁴ IVS-0020 Clonal Control RNA
4-089-2800	IVS-0032 Clonal Control RNA
4-089-2810	10 ⁻¹ IVS-0032 Clonal Control RNA

4-089-2820 10⁻² IVS-0032 Clonal Control RNA
4-089-2830 10⁻³ IVS-0032 Clonal Control RNA
4-089-2840 10⁻⁴ IVS-0032 Clonal Control RNA
4-089-2850 10⁻⁵ IVS-0032 Clonal Control RNA
4-089-2860 10⁻⁶ IVS-0032 Clonal Control RNA
4-089-3070 IVS-0035 Clonal Control RNA

Tissue DNA Control

4-092-0010 IVS-0000 Polyclonal Control DNA

RNA Proficiency Panel

4-310-0100 Proficiency Panel for *BCR/ABL* t(9;22) Translocations

LymphoTrack® Low Positive Controls

4-088-0098 LymphoTrack® B-cell Low Positive Control
4-088-0108 LymphoTrack® T-cell Low Positive Control

LymphoQuant® Internal Controls

4-088-0118 LymphoQuant® B-cell Internal Control
4-088-0128 LymphoQuant® T-cell Internal Control

Somatic Hypermutation Sanger Sequencing Assays

5-101-0030 *IGH* Somatic Hypermutation Assay v2.0 – Gel Detection
5-101-0031 *IGH* Somatic Hypermutation Assay v2.0 – ABI Fluorescence Detection
5-101-0040 *IGH* Somatic Hypermutation Assay v2.0 MegaKit – Gel Detection
5-101-0041 *IGH* Somatic Hypermutation Assay v2.0 MegaKit – ABI Fluorescence

ABI Reagents

6-098-0051 HI-Deionized Formamide with ROX Size Standard (ABI 310)
6-098-0061 HI-Deionized Formamide with ROX Size Standard (ABI 3100)

Taq DNA Polymerases

6-097-0100 EagleTaq DNA Polymerase
6-097-0130 FalconTaq DNA Polymerase

Next-Generation Sequencing CE-IVD LymphoTrack® Dx Assays

9-121-0007 LymphoTrack® Dx *IGH* FR1 Assay – S5/PGM™
9-121-0009 LymphoTrack® Dx *IGH* FR1 Assay Kit A – MiSeq™
9-121-0037 LymphoTrack® Dx *IGH* FR2 Assay – S5/PGM™
9-121-0039 LymphoTrack® Dx *IGH* FR1 Assay Panel – MiSeq™
9-121-0047 LymphoTrack® Dx *IGH* FR3 Assay – S5/PGM™
9-121-0057 LymphoTrack® Dx *IGH* FR1/2/3 Assay – S5/PGM™
9-121-0059 LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assay Kit A – MiSeq™
9-121-0069 LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assay Panel – MiSeq™
9-121-0089 LymphoTrack® Dx *IGH* FR2 Assay Kit A – MiSeq™
9-121-0099 LymphoTrack® Dx *IGH* FR2 Assay Panel – MiSeq™
9-121-0109 LymphoTrack® Dx *IGH* FR3 Assay Kit A – MiSeq™
9-121-0119 LymphoTrack® Dx *IGH* FR3 Assay Panel – MiSeq™
9-121-0129 LymphoTrack® Dx *IGH* FR1/2/3 Assay Kit A – MiSeq™
9-121-0139 LymphoTrack® Dx *IGH* FR1/2/3 Assay Panel – MiSeq™
9-122-0009 LymphoTrack® Dx *IGK* Assay Kit A – MiSeq™
9-122-0019 LymphoTrack® Dx *IGK* Assay Panel – MiSeq™
9-122-0007 LymphoTrack® Dx *IGK* Assay – S5/PGM™
9-225-0009 LymphoTrack® Dx *TRB* Assay Kit A – MiSeq™
9-225-0019 LymphoTrack® Dx *TRB* Assay Panel – MiSeq™
9-227-0007 LymphoTrack® Dx *TRG* Assay – S5/PGM™
9-227-0009 LymphoTrack® Dx *TRG* Assay Panel – MiSeq™
9-227-0019 LymphoTrack® Dx *TRG* Assay Kit A – MiSeq™
9-500-0007 LymphoTrack® Dx Software – S5/PGM™
9-500-0009 LymphoTrack® Dx Software – MiSeq™

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Intended Uses

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Patent Notice

Many Many of the products described herein are covered by one or more patents including European Patent No. 1549764, European Patent No. 2418287, European Patent No. 2460889, European Patent No. 1633884, Japanese Patent No. 4708029, United States Patent No. 7,785,783, United States Patent No. 8859748 and United States Patent No. 10280462, Japanese Patent No. 6189600, Brazilian Patent No. PI0410283, Canadian Patent No. 2525122, Indian Patent No. 243620, Mexican Patent No. 286493, Chinese Patent No. 1806051, and Korean Patent No. 10-1215194. All of these patents are licensed to Invivoscribe®.

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