LymphoTrack® IGH Somatic Hypermutation Assay - MiSeq, Product Flyer

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Assay Uses

This research use only (RUO) assay identifies clonal IGH V-J rearrangements, the associated V-J region DNA sequences, provides the frequency distribution of V region and J region segment utilization using the Illumina MiSeq® platform and defines the extent of somatic hypermutation present in the IGHV gene of analyzed samples.

Background

The immunoglobulin heavy chain (IGH) gene locus on chromosome 14 (14q32.3) includes 46-52 functional and 30 non-functional variable (VH) gene segments, 27 functional diversity (DH) gene segments, and 6 functional joining (JH) gene segments spread over 1250 kilobases.

Lymphoid cells are different from the other somatic cells in the body as during development, the antigen receptor genes in lymphoid cells undergo somatic gene rearrangement (Tonegawa S. et al., 1983). During B cell development, genes encoding the immunoglobulin heavy chain (IGH) molecules are assembled from multiple polymorphic gene segments that undergo rearrangements and selection. These gene rearrangements of the VH, DH, and JH segments generate VH-DH-JH combinations of unique length and sequence for each cell. An additional level of diversity is generated by point mutations in the variable regions, somatic hypermutations (SHM). 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.

References


Specimen Requirement

50 ng of genomic DNA.

Method

This LymphoTrack IGH Somatic Hypermutation Assay represents a significant improvement over existing clonality assays using fragment analysis as it efficiently detects the majority of IGH gene rearrangements using a single multiplex master mix and, at the same time, the assay identifies the DNA sequence specific for each clonal gene rearrangement.

Therefore, this product has two important and complementary uses: it aids both in the detection of initial clonal populations, and identifies sequence information required to track those clones in subsequent samples and to determine the SHM status of samples.

Our single multiplex master mix targets the conserved leader sequence upstream of framework 1 (FRI) and the joining (J) region. Primers included in the master-mix are designed with Illumina adapters and 24 different indices. This allows for a one-step PCR reaction and pooling of amplicons from several different samples for loading on the MiSeq flow cell.

Positive and negative controls are included in the kit.

Ordering Information

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Products</th>
<th>Quantity</th>
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</thead>
<tbody>
<tr>
<td>7-121-0059</td>
<td>LymphoTrack IGH Somatic Hypermutation Assay Kit A - MiSeq</td>
<td>8 indices - 5 reactions each</td>
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<tr>
<td>7-121-0069</td>
<td>LymphoTrack IGH Somatic Hypermutation Assay Panel - MiSeq</td>
<td>24 indices - 5 reactions each</td>
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<tr>
<td>7-121-0079</td>
<td>LymphoTrack IGH SHM MiSeq Software*</td>
<td>1 CD</td>
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</table>

*only available with purchase of a LymphoTrack IGH Somatic Hypermutation MiSeq Assay

This product is sold FOR RESEARCH USE ONLY, not for use in diagnostic procedures.
Figure 1: Simplified representation of the organization of the immunoglobulin heavy chain (IGH) gene on chromosome 14. Depicted are the variable region (VH) genes and downstream consensus joining region genes (JH) that are involved in rearrangements.

Figure 2: Example data of a read summary with mutation rate from a MiSeq run is depicted. Amplicons were generated from a CLL blood specimen using an IGH Leader MiSeq Master Mix. Data was analyzed using the LymphoTrack® IGH SHM MiSeq Software package. Excerpt of the Read summary including Mutation rate to partial V gene (%), In-frame mutation or Stop codons found and V-Coverage, showing the top read broken down for visibility into columns 1-5 (A), columns 6-9 (B), and columns 10-12 (C).