LeukoStrat®
FLT3 Mutation Assaty 2.0

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Intended Use

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:

- Identify internal tandem duplications (ITD) in the *FLT3* gene
- Identify tyrosine kinase domain (TKD) mutations in the *FLT3* gene

Summary

AML in general has a poor prognosis[1,2]. Assessment of the mutation status of the *FLT3* (fms related tyrosine kinase 3) receptor gene is the most important prognostic indicator of disease outcome, occurring in approximately 30% of patients at the time of diagnosis[3]. For this reason, testing for *FLT3* activating mutations is required for the stratification of disease and determination of appropriate treatment options. The LeukoStrat® *FLT3* Mutation Assay 2.0 is a PCR-based method that identifies internal tandem duplication (ITD) mutations and tyrosine kinase domain (TKD) mutations.

This test kit includes 2 master mixes: the *FLT3*-ITD Master Mix for the detection of ITD mutations and *FLT3*-D835 Master Mix for the detection of TKD region mutations (such as the D835 and I836 mutations).

Performance Characteristics

The LeukoStrat® *FLT3* Mutation Assay 2.0 is a rapid and reliable method for the detection of *FLT3* mutations, as evidenced by comparison with Roche® 454 sequencing.

The *FLT3* Mutation Assay 2.0 is capable of detecting *FLT3*-ITD and TKD mutations with an excellent concordance (Table 1 and 2) and has high reliability when multiple standard laboratory variables are considered including multiple operators, reagent lots, different ABI 3500xL instruments, and nonconsecutive testing days.

Table1. *FLT3* ITD Percent Agreement 454 Sequencing

Negative Percent Agreement		Discordance #	Concordance #	*95% LL
Negative PA	100%	0	119	96.9%
Positive 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).

Table2. *FLT3* TKD Percent Agreement with 454 Sequencing

Negative Percent Agreement		Discordance #	Concordance #	*95% LL
Negative PA	100%	0	137	96.9%
Positive 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., (2003). Detection of FLT3 Internal Tandem Duplication and D835 Mutations by a Multiplex Polymerase Chain Reaction and Capillary Electrophoresis Assay. *The Journal of Molecular Diagnostics* 5, 96 – 102.
2. Yamamoto, Y. et al., (2001). Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. *Blood* 97, 2434-2439.
3. Acute Myeloid Leukemia, Clinical Practice Guidelines in Oncology, (v.2.2014) National Comprehensive Cancer Network.

Ordering information

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

CE IVD These are *in vitro* diagnostic products, and are not available for sale or use within North America.

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Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0017 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	1 x 100 µL tubes
IVS-P004 Clonal Control DNA	171 pg/mL	1 x 100 µL tube	1 x 100 µL tubes
IVS-0009 Clonal Control DNA	200 µg/mL	3 x 100 µL tube	5 x 100 µL tubes
Master Mixes	**Target**	**Units in Assay**	**Units in Assay MegaKit**
FLT3 ITD Master Mix – 6FAM & HEX	*FLT3* ITD	1 x 1500 µL tube	10 x 1500 µL tubes
FLT3 D835 Master Mix – 6FAM	*FLT3* TKD	1 x 1500 µL tube	10 x 1500 µL tubes

Principles of the Procedure

FLT3 ITD or length mutations are caused by duplication and insertion of a portion of the *FLT3* gene that includes the region in and around the juxtamembrane (JM) region. Detection of ITD mutations is determined by the increased size of the PCR products (larger than 330 bp) compared to the products produced by wild-type genes.

FLT3 TKD mutations are caused by nucleic acid substitutions that result in a change in the amino acid sequence in the highly conserved catalytic center. Detection of D835 mutations requires enzymatic digestion with EcoRV (not included). Wild-type alleles of the *FLT3* gene, yield products of 79 bp and mutant alleles yield products of 127 bp. Undigested amplicons are 147 bp.

This assay can reliably detect mutations comprising more than 5% of the total cell population.

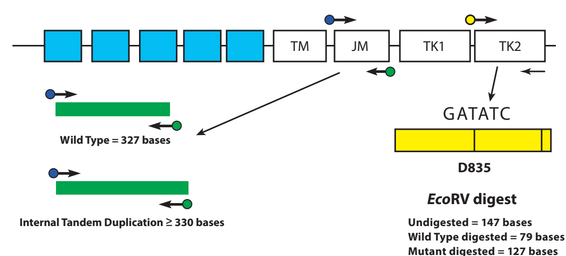
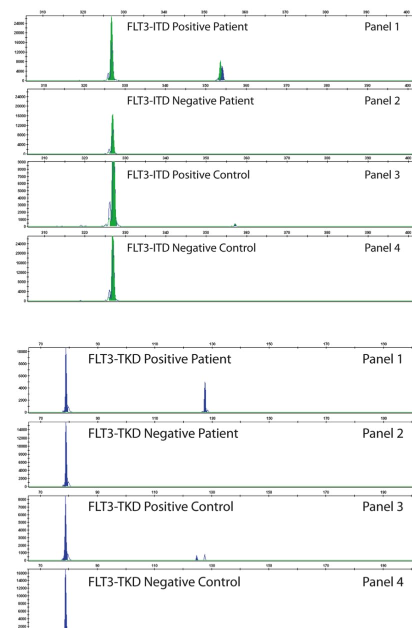


Figure 1. Depicted is a representation of the *FLT3* JM region and the activating loop of the kinase domain. Green and blue dots with black arrows represent the relative positions of primers that target the JM region for ITD and yellow dots with black arrows represent the relative positions of the primers that target TKD mutations in the activating loop of the kinase domain. The yellow box has vertical black lines that represent the position of the wild-type EcoRV restriction digest sites.

Sample Data

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



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For additional patent information, contact our legal department by email at legal@invivoscribe.com, or by telephone in the United States: 1 (858) 224-6600.

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