LeukoStrat®
FLT3 Mutation Assay 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

AML

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[1,2]. For this reason *FLT3* mutation testing is required to stratify disease and determine appropriate treatment options.

> Each year approximately 21,000 patients in the United States are diagnosed with acute myeloid leukemia (AML). Of those diagnosed with AML, ~1 out of 3 are expected to have presence of *FLT3* mutations, (*FLT3*mut+)[3].

Test Methodology

This PCR-based assay targets regions of the *FLT3* gene to identify ITD mutations and TKD mutations (such as the D835 and I836 mutations) in sample human genomic DNA. DNA is amplified by PCR with fluorophore-labeled primers, TKD amplicon is enzymatically digested, and *FLT3* mutations are detected via capillary electrophoresis.

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

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).

Table 2. *FLT3* TKD Percent Agreement with 454 Sequencing

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

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Reagents

Controls	Concentration	Units in Assay	Units in Assay MegaKit
FLT3 ITD Postive Control	50 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
FLT3 D835 Postive Control	50 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
FLT3 Negative Control	50 µg/mL	1 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 327 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 ~124/127 bp. Undigested amplicons are 147 bp.

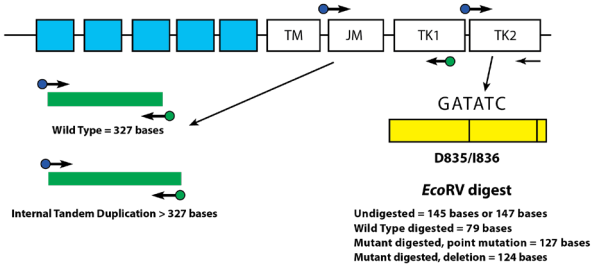


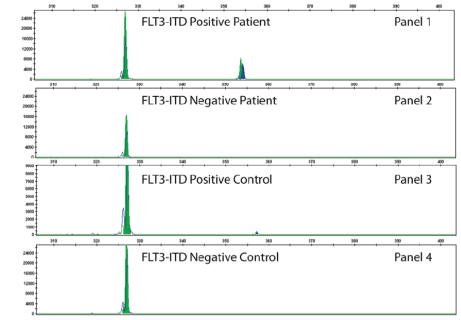
Figure 1. Depicted is a representation of the *FLT3* JM region and the activating loop of the kinase domain. Green and blue dots with black arrows represent the relative positions of primers that target in and around the JM region for ITD. The blue dot and black arrow on the TKD region represent the 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.

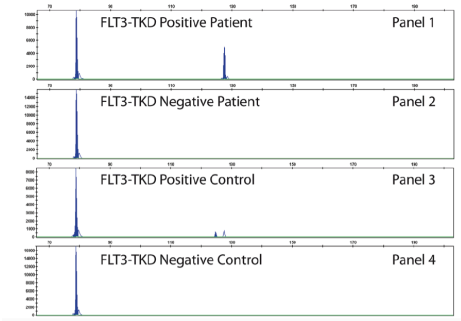
Product sizes reflect human gDNA templates.

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. 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 kit provided controls and master mixes. Amplified products were run on an ABI 3500xL instrument.





This product is an in vitro diagnostic product; not available for sale or use within North America.

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