FLT3 Mutation Assays

Assay Use

This research use only (RUO) assay identifies *FLT3* Mutations.

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).

Test Methodology

FLT3 Mutation Assays target 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, TKD amplicon is enzymatically digested, and *FLT3* mutations are detected via gel (catalog #14120010, #14120020) or capillary electrophoresis (catalog #14120031, #14120041).

Product Configuration

Test kits include three PCR master mixes, along with positive and negative controls. *FLT3* ITD master mix targets internal tandem duplication mutations. *FLT3* D835 master mix targets 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.

References

1. *NEJM.* 2013; 368(22): 2059-74)

2. *Blood.* 2002; 99(12): 4326-35.

3. *Expert Opin. Ther. Targets.* 2015; 19(1): 37-54

4. *Blood.* 2001; 97:2434-2439.

5. https://clinicaltrials.gov/

Ordering information

Catalog #	Products	Quantity
1-412-0010	*FLT3* Mutation Assay - Gel Detection	33 reactions
1-412-0020	*FLT3* Mutation Assay MegaKit - Gel Detection	330 reactions
1-412-0031	*FLT3* Mutation Assay - ABI Fluorescence Detection	33 reactions
1-412-0041	*FLT3* Mutation Assay MegaKit - ABI Fluorescence Detection	330 reactions

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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	5 x 100 µL tubes
IVS-P001 Clonal Control DNA	200 pg/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**
FLT3 ITD Master Mix	*FLT3* ITD	1 x 1500 µL tube	10 x 1500 µL tubes
FLT3 D835 Master Mix	*FLT3* TKD	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

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

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 of the *FLT3* gene. As ITD mutations vary in both the location and the length of the inserted duplicated DNA sequence, their detection 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 this highly conserved catalytic center. When a nucleic acid substitution occurs, the wild type EcoRV recognition site disappears, and EcoRV endonuclease is unable to identify and digest the DNA at this site. Using this assay, the *FLT3* TKD region is PCR amplified, digested with EcoRV, and analyzed by capillary or gel electrophoresis. To control for digestion one of the PCR primers contains an EcoRV restriction site. Thus, the digestion pattern identifies loss of the normal gene sequence while further ensuring that digestion occurred.

See figure 1 for further details.

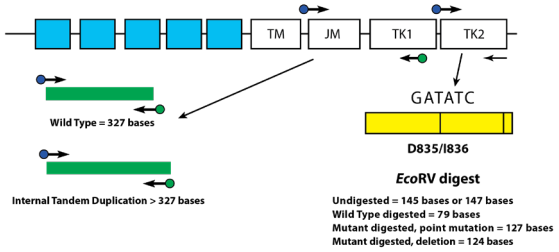


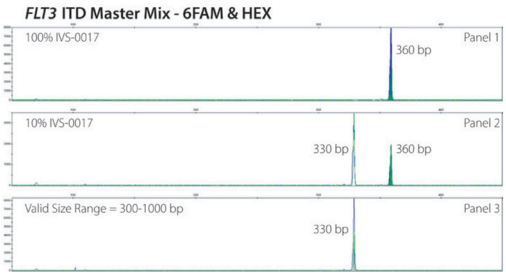
Figure 1 (above): Depicted is a representation of the *FLT3* JM region and the activating loop of the kinase domain. Green and blue dots with black arrows represent the relative positions of primers that target in and around the JM region for ITD. The blue dot and black arrow on the TKD region represent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

DNA templates amplified with *FLT3* ITD Master Mix were analyzed by gel (on a 2% agarose TBE gel alongside a 100bp DNA size ladder) or capillary electrophoresis (on an ABI 3100 platform). Lane1/Panel 1 is a *FLT3* ITD positive control*; lane 2/panel 2 is a 10% dilution of the positive control; and Lane 3/Panel 3 is IVS-0000, which is representative of a wild type product.

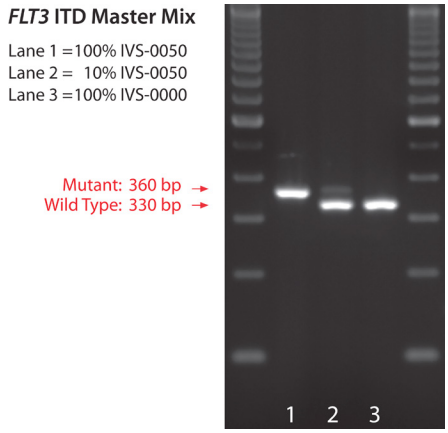
*IVS-0017 is provided in kits as the *FLT3* ITD positive control DNA. IVS-0050 template performs comparable to IVS-0017.



« Capillary Detection

FLT3 ITD Master Mix

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



» Gel Detection