# LeukoStrat®
## CDx *FLT3* Mutation Assay

## The only internationally standardized CE-IVD assay for *FLT3* Signal Ratio mutation analysis.

For selection of acute myeloid leukemia (AML) patients eligible for treatment with midostaurin.

For illustrative purposes only.

### Intended Use

The LeukoStrat CDx *FLT3* Mutation Assay is a PCR-based *in vitro* diagnostic test designed to detect internal tandem duplications (ITD) *FLT3* mutations and tyrosine kinase domain (TKD) *FLT3* mutations 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 for whom midostaurin treatment is being considered.

### Background

Primers flanking exons 14, 15 and the activation loop region of exon 20 of the *FLT3* gene are used to amplify DNA extracted from a patient sample. The forward and reverse PCR primers are fluorescently labeled with different fluorophores that serve to confirm the presence of sample signal.

The size of the ITD PCR product is determined by capillary electrophoresis and the signal ratio (SR) compares the signal intensity of the mutant to the wild-type. *FLT3* TKD PCR product is digested with EcoRV and the presence of the mutation is further assessed using capillary electrophoresis and the signal ratio (SR) compares the signal intensity of the mutant to the wild-type.

The *FLT3* Mutation Assay includes reagents, software, and procedures for isolating mononuclear cells and extracting DNA from patient specimens to determine if *FLT3* mutations are present.

### Specimen Requirement

At least 1 mL of peripheral blood or 0.25 mL of bone marrow anti-coagulated with sodium heparin.

### Method

#### Internal Tandem Duplication (ITD) Mutations of *FLT3*

The LeukoStrat CDx *FLT3* Mutation Assay uses fluorescently labeled primers that are in 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 (Figure 1).

#### Tyrosine Kinase Domain (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 (Figure 1).

### References

1. Murphy KM, et al. Detection of FLT3 Internal Tandem Duplication and D835 Mutations by a MultiplePolymerase Change Reaction and Capillary Electrophoresis Assay. *Journal of Molecular Diagnostics* 2003, 5:96-102.

2. Yamamoto, Y, et al. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. *Blood*, 2001, 97(8):2434-9.

### Ordering Information

| CATALOG # | PRODUCTS | QUANTITY |
|---|---|---|
| K-412-0291 | LeukoStrat® CDx *FLT3* Mutation Assay | 33 reactions |
| K-412-0281 | LeukoStrat® CDx *FLT3* Mutation Assay Software | 1 CD complimentary with purchase |

This product is a CE-IVD assay for *In Vitro* diagnostic use.

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## Principle of *FLT3* Mutation Analysis

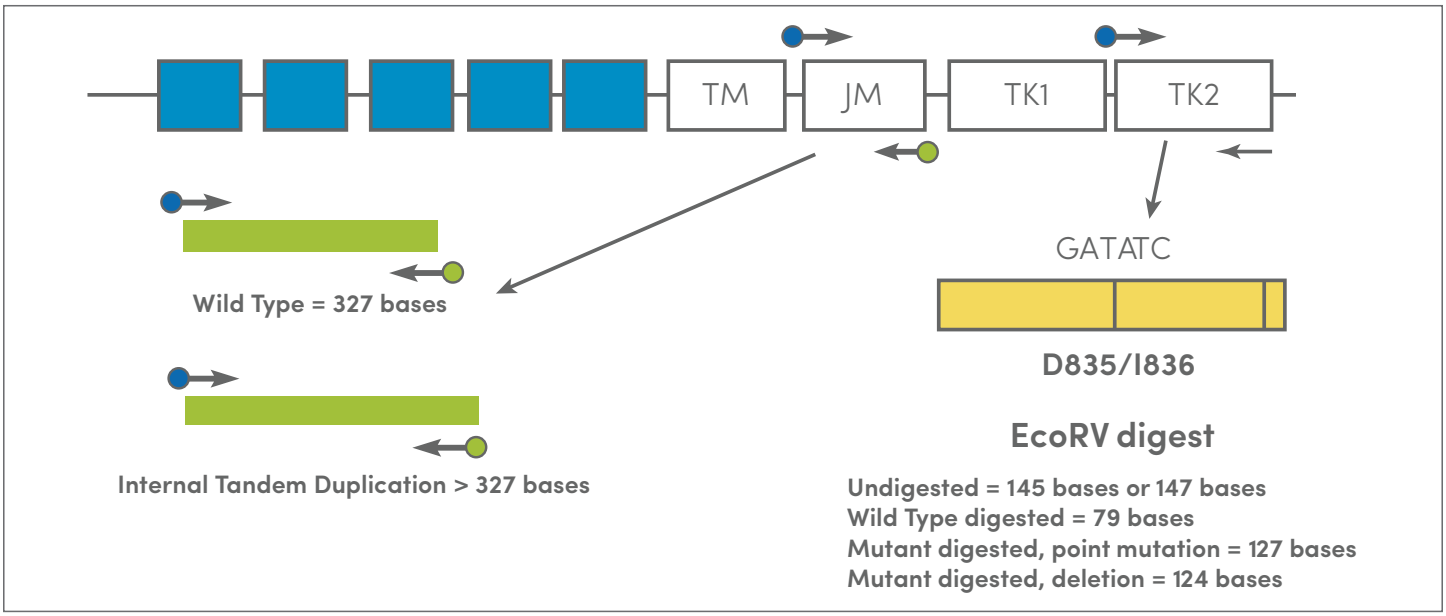


Figure 1: Depicted is a representation of the *FLT3* juxtamembrane (JM) region (TM = transmembrane) and the activating loop of the tyrosine kinase (TK) domain. Black arrows represent the relative positions of primers that target the JM region for ITD or the activating loop of the kinase domain for TKD. 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 | Unit Quantity |
|---|---|
| *FLT3* Extraction Control | 1800 µL |
| *FLT3* ITD Master Mix | 1500 µL |
| *FLT3* TKD Master Mix | 1500 µL |
| *FLT3* ITD Positive Control | 100 µL |
| *FLT3* TKD Positive Control | 100 µL |
| *FLT3* No Template Control | 200 µL |

All reagents should be stored at -15 to -30 degrees C.

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CE-marked *in vitro* diagnostics are not available for sale or use in North America.