LeukoStrat®

CDx *FLT3* Mutation Assay (IVD)

FDA APPROVED COMPANION DIAGNOSTIC

In Vitro Diagnostic Kit
PN: K4120361

FDA Approved Assay for assessment of acute myeloid leukemia (AML) patients eligible for treatment with RYDAPT® (midostaurin) or XOSPATA® (gilteritinib fumarate), **Now Available as US Distributed Kit**. This *FLT3* companion diagnostic includes reagents along with software that identifies ITD and TKD mutations, generates mutant/wildtype signal ratios, and predicts response to gilteritinib and midostaurin.

Clinical Significance of *FLT3* Mutation Status

Each year approximately 21,000 patients in the United States are diagnosed with AML. Of those diagnosed with AML, ~1 out of 3 are expected to have presence of *FLT3* mutations, (*FLT3*mut+). Since *FLT3*mut+ AML is clinically actionable, stratification of AML patients by testing for *FLT3* mutation status has become a standard of care.

Intended Use

The LeukoStrat CDx *FLT3* Mutation Assay is a PCR-based *in vitro* diagnostic test designed to detect internal tandem duplication (ITD) and tyrosine kinase domain (TKD) mutations D835 and I836 in the *FLT3* gene in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myelogenous leukemia (AML).

The LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom RYDAPT® (midostaurin) treatment is being considered.

The LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom XOSPATA® (gilteritinib) treatment is being considered.

The test is for use on the 3500xL Dx Genetic Analyzer.

Summary and Explanation of the Test

Acute myelogenous leukemia (AML) in general has a poor prognosis. Many studies in AML have shown that the presence of *FLT3* activating mutations portends a poor prognosis making it an attractive target for treatment.[1,2]

The LeukoStrat CDx *FLT3* Mutation Assay targets regions of the *FLT3* gene to identify internal tandem duplication (ITD) mutations and tyrosine kinase domain (TKD) mutations, such as the D835 and I836 mutations.

The LeukoStrat CDx *FLT3* Mutation Assay includes reagents, software and procedures for isolating mononuclear cells and extracting DNA from patient peripheral blood or bone marrow specimens to determine if *FLT3* mutations are present.

DNA is amplified via PCR and the amplicons are detected via capillary electrophoresis. *FLT3* mutation status is determined by the LeukoStrat CDx *FLT3* Software. A *FLT3* ITD and/or TKD mutation is reported as Positive if the mutant:wild-type signal ratio meets or exceeds the clinical cutoff of 0.05.

Method Description

ITD Mutations of *FLT3*

The LeukoStrat CDx *FLT3* Mutation Assay uses fluorescently labeled primers that are in and around 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 (schematic on back page).

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 (schematic on back page).

Reference

1. Murphy KM et al., A Clinical PCR/Capillary Electrophoresis Assay for the Detection of Internal Tandem Duplication and Point Mutation of the FLT3 Gene. J. Mol. Diag. 5:96-102 (2003).

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

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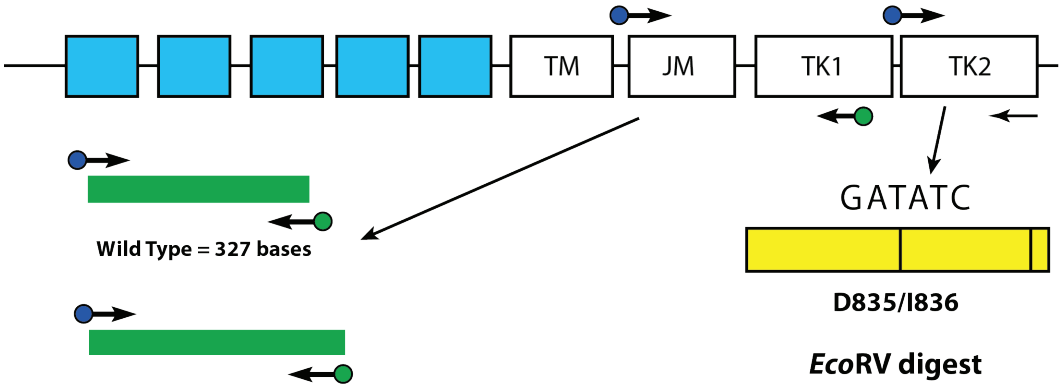
Method Schematic: *FLT3* ITD & TKD Mutant Detection



***Eco*RV digest**

Undigested = 145 bases or 147 bases
Wild Type digested = 79 bases
Mutant digested, point mutation = 127 bases
Mutant digested, deletion = 124 bases

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 in and around 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	Units in Assay
FLT3 Extraction Control	1 x 1800 µL tube
FLT3 ITD Master Mix	1 x 1500 µL tube
FLT3 TKD Master Mix	1 x 1500 µL tube
FLT3 ITD Positive Control	1 x 100 µL tube
FLT3 TKD Positive Control	1 x 100 µL tube
FLT3 No Template Control	1 x 200 µL tube
Taq DNA Polymerase	1 x 200 µL tube
EcoRV Enzyme	1 x 200 µL tube
NEBuffer 3.1	1 x 1250 µL tube

Ordering Information

Catalog #	Products	Quantity
K-412-0361	LeukoStrat® CDx *FLT3* Mutation Assay	33 Reactions
K-412-0371	LeukoStrat® CDx *FLT3* Software	CD complimentary with purchase
K-412-0401	LeukoStrat® CDx Assay Installer	USB complimentary with purchase

IVD These are *in vitro* diagnostic (IVD) products, and are available for sale or use in the United States only.

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