



Molecular Diagnostic Laboratory  
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## **FLT3 Gene Internal Tandem Duplication and D835 Mutations**

Internal tandem duplication (ITD) and D835 mutations in the *FLT3* gene of some patients with Acute Myeloid Leukemia (AML)

### **Indications for Molecular Testing**

- Patients diagnosed with acute myeloid leukemia AML.
- At least 10% of the cells in the specimen are leukemia cells.

### **Testing Methodology**

Polymerase chain reaction (PCR), followed by capillary gel electrophoresis (fragment sizing) for internal tandem duplication (ITD) mutations and restriction enzyme digestion with Eco RV for identification of the D835 mutation. (PCR is utilized pursuant to a license agreement with Roche Molecular Systems, Inc.)

### **Interpretation of DNA analysis**

There are two specific *FLT3* mutations that have important implications for acute myeloid leukemia (AML) prognosis. The first mutation is an internal tandem duplication (ITD) mutation. This alteration arises from duplications of the juxtamembrane portion of the gene (exons 11 & 12), to produce activation of the FLT3 protein (AML). This alteration has been identified in 20% to 30% of patients with acute myelogenous leukemia. The second type of *FLT3* mutation is a missense mutation at aspartic acid residue 835 (D835) within the activation loop of the FLT3 protein. This alteration occurs in about 7% of AML cases. Both mutations activate the FLT3 protein and appear to be associated with a poor prognosis in patients with AML including decreased survival, decreased disease-free survival, and increased risk of relapse.

### **Specimen Requirements**

**Frozen Tissue**--10 mm<sup>3</sup> of fresh frozen tissue in sterile, plastic container. Forward frozen tissue on dry ice.  
**Formalin-Fixed, Paraffin-Embedded (FFPE) Tissue**--Twenty 10 micron sections of FFPE tissue in a sterile, microcentrifuge tube. **Peripheral Blood**--1 lavender-top (EDTA) tube. Invert several times to mix blood. **Bone Marrow**--Place 1-2 mL of anticoagulated bone marrow in a lavender-top (EDTA) tube. Invert several times to mix bone marrow. Do not freeze blood, bone marrow, or FFPE, forward promptly at ambient temperature to the following address:

**Molecular Diagnostic Laboratory**  
**Barnes-Jewish Hospital North, Room 2445**  
**Mail Stop 90-28-372**  
**216 South Kingshighway**  
**St. Louis, MO 63110**

Clinical information must be provided with specimen referral in order to correctly interpret test results.

### **Current Pricing**

Contact Lab Customer Service for current pricing 314 362-1470.  
CPT codes: 83907, 83890, 83892, 83894, 83898, and 83912.

Murphy, Lewis, Hafez, Geiger, Cooper, Smith, Small, and Berg. *Detection of FLT3 Internal Tandem Duplication and D835 Mutations by a Multiplex Polymerase Chain Reaction and Capillary Electrophoresis Assay*. Journal of Molecular Diagnostics, Vol. 5, No. 2 May 2003

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Rombouts et al. Biological characteristics and prognosis of adult acute myeloid leukemia with internal tandem duplications in the FLT3 gene. Leukemia 2000;14(4):675-683