

FLT3 (F594_R595insR), Active

Recombinant human protein expressed in Sf9 cells

Catalog # F12-12EG

Lot # P1869-9

Product Description

Recombinant human FLT3 (F594_R595insR) (571-993) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_004119](#). This FLT3-ITD mutant has amino acid "R" repeated at the site R595 within the juxtamembrane domain.

Gene Aliases

FLT3 (F594_R595insR), FLT3-ITD, FLK2, STK1, CD135

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

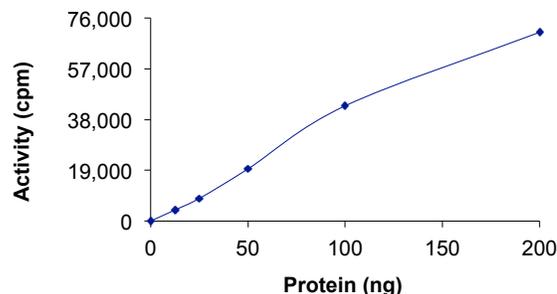
Scientific Background

Fms-related tyrosine kinase 3 (FLT3) is a gene that encodes for a tyrosine kinase that activates pathways in hematopoietic cells important in cellular proliferation. FLT3 is frequently mutated in acute myeloid leukemia (AML), myelodysplastic syndromes, other hematologic malignancies, and colorectal cancer. FLT3 mutations are observed in 24.3% of AML, of which internal tandem duplications (ITD) are the most frequent. FLT3 ITD occurs when sequences of less than ten to several hundred bases in length are repeated within the juxtamembrane domain are always "in-frame".

References

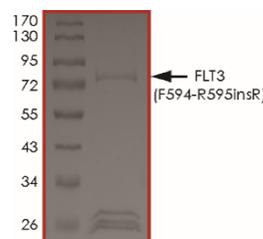
1. <http://www.mycancergenome.org/content/disease/acute-myeloid-leukemia/flt3/277/>
2. Fathi AT, et al: Treatment of FLT3-ITD acute myeloid leukemia. Am J Blood Res. 1(2):175-89, 2011

Specific Activity



The specific activity of FLT3 (F594_R595insR) was determined to be **20 nmol/min/mg** as per activity assay protocol.

Purity



The purity of FLT3 (F594_R595insR) was determined to be **>70%** by densitometry, approx. MW **73 kDa**.

FLT3 (F594_R595insR), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	F12-12EG
Specific Activity	20 nmol/min/mg
Lot #	P1869-9
Purity	>70%
Concentration	0.05 µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: F12-12EG)

Active FLT3 (F594_R595insR) (0.05 μ g/ μ l) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active FLT3 (F594_R595insR) for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/ μ l BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β -glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250 μ M [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 μ l of 10mM ATP Stock Solution (Catalog #: A50-09), 100 μ l [³³P]-ATP (1mCi/100 μ l), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200 μ l aliquots at -20°C.

Substrate (Catalog #: M42-51N)

Myelin basic protein (MBP) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active FLT3 (F594_R595insR), Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 μ l:
 - Component 1.** 10 μ l of diluted Active FLT3 (F594_R595insR) (Catalog #F12-12EG)
 - Component 2.** 5 μ l of 1mg/ml stock solution of substrate (Catalog #M42-51N)
 - Component 3.** 5 μ l distilled H₂O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5.** Initiate the reaction by the addition of 5 μ l [³³P]-ATP Assay Cocktail bringing the final volume up to 25 μ l and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 μ l of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μ l [³³P]-ATP / pmoles of ATP (in 5 μ l of a 250 μ M ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/ μ g or nmol/min/mg)

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μ g or mg)]*[(Reaction Volume) / (Spot Volume)]

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