

# FLT3 Kinase

✓ 5 µl



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This product is for *in vitro* research use only and is not intended for use in humans or animals.

**Description:** Purified recombinant human FLT3 (Arg571-Ser993) kinase, supplied as a GST fusion protein.

**Background:** FMS-related tyrosine kinase 3 (FLT3, also called Flk2), is a member of the type III receptor tyrosine kinase family, which includes c-Kit, PDGFR and M-CSF receptors. FLT3 is expressed on early hematopoietic progenitor cells and supports growth and differentiation within the hematopoietic system (1,2). FLT3 is activated after binding with its ligand FL, which results in a cascade of tyrosine autophosphorylation and tyrosine phosphorylation of downstream targets (3). The p85 subunit of PI3 kinase, SHP2, GRB2 and Shc are associated with FLT3 after FL stimulation (4-6). Tyr589/591 is located in the juxtamembrane region of FLT3 and may play an important role in regulation of FLT3 tyrosine kinase activity. Somatic mutations of FLT3 consisting of internal tandem duplications (ITDs) occur in 20% of patients with acute myeloid leukemia (7).

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human FLT3 (Arg571-Ser993) (GenBank Accession No. NM\_004119) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

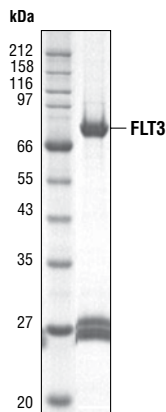


Figure 1. The purity of the GST-FLT3 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

**Quality Control:** The theoretical molecular weight of the GST-FLT3 fusion protein is 73 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. FLT3 kinase activity was determined using a radiometric assay [Fig.2].

#### Background References:

- (1) Shurin, M.R. et al. (1998) *Cytokine Growth Factor Rev.* 9, 37–48.
- (2) Naoe, T. et al. (2001) *Cancer Chemother. Pharmacol.* 48 Suppl1, S27–S30.
- (3) Namikawa, R. et al. (1996) *Stem Cells* 14, 388–395.
- (4) Beslu, N. et al. (1996) *J. Biol. Chem.* 271, 20075–20081.
- (5) Zhang, S. and Broxmeyer, H.E. (2000) *Biochem. Biophys. Res. Commun.* 277, 195–199.
- (6) Zhang, S. et al. (1999) *J. Leukoc. Biol.* 65, 372–380.
- (7) Mizuki, M. et al. (2000) *Blood* 96, 3907–3914.

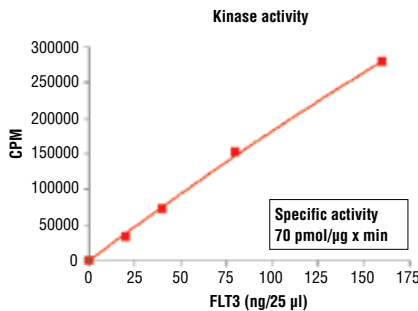


Figure 2. FLT3 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM MgCl<sub>2</sub>, 0.05 mM DTT, 50 µM ATP, Substrate: MBP 200 ng/µL, and variable amounts of FLT3.

**Storage:** Enzyme is supplied in 50 mM Tris-HCl, pH7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

#### Companion Products:

HTScan® Tyrosine Kinase Buffer (4X) #9805

ATP (10 mM) #9804

Tyrosine Kinase Substrate Screening Kit #7450

# Protocol for Flt3 Kinase Assay

**Note:** Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

## A Additional Solutions and Reagents (Not included)

### 1. Kinase Buffer (10X)

- 50 mM MOPS, pH 7.2
- 25 mM  $\beta$ -glycerophosphate
- 10 mM EGTA
- 4 mM EDTA
- 50 mM  $MgCl_2$
- 0.5 mM DTT

### 2. ATP (10 mM) #9804

### 3. $^{32}P$ - $\gamma$ ATP

### 4. MBP (0.5 $\mu$ g/ $\mu$ l)

## B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250  $\mu$ M ATP.
2. Dilute [ $^{32}P$ ] ATP to 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP with 250  $\mu$ M ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute Flt3 protein (100 ng/ $\mu$ l concentration) to 20 ng/ $\mu$ l with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10  $\mu$ l diluted Flt3 kinase solution, 10  $\mu$ g MBP (0.5  $\mu$ g/ $\mu$ l), and 5  $\mu$ l 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP solution.

## Final Assay Conditions

- 5 mM MOPS, pH 7.2
  - 2.5 mM  $\beta$ -glycerophosphate
  - 1 mM EGTA
  - 0.4 mM EDTA
  - 5 mM  $MgCl_2$
  - 0.5 mM  $CaCl_2$
  - 200 ng/ $\mu$ l MBP
6. After 15 minutes terminate reaction by spotting 20  $\mu$ l of the reaction mixture onto phosphocellulose P81 paper.
  7. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
  8. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
  9. Count samples in a scintillation counter.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: [drugdiscovery@cellsignal.com](mailto:drugdiscovery@cellsignal.com).