Store at -80°C

#7742

FLT3 Kinase

☑5 µg

Cell Signaling TECHNOLOGY®

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Description: Purified recombinant human FLT3 kinase (Arg571-Ser993), 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. It 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).

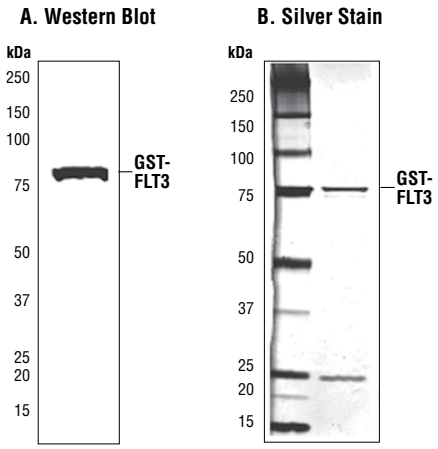

Figure 1. The purity of the GST-FLT3 fusion protein was analyzed using SDS/PAGE followed by anti-FLT3 Western blot (A) or Silver stain (B).

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.

Quality Control: The theoretical molecular weight of the GST-FLT3 fusion protein is 82 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Silver stain and Western blot (Fig.1). FLT3 kinase activity was determined using a radiometric assay (Fig.2). A kinase dose dependency assay was performed to measure FLT3 activity using HTScan™ FLT3 Kinase Assay Kit 7743 (Fig.3).

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.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 8.0; 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol. Store at –80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

HTScan™ FLT3 Kinase Assay Kit #7743

Gastrin Precursor (Tyr87) Biotinylated Peptide #1310

Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411

HTScan™ Tyrosine Kinase Buffer (4X) #9805

ATP (10 mM) #9804

Staurosporine #9953

Tyrosine Kinase Substrate Screening Kit #7450

HTScan™ Profiling Kit (Tyrosine Kinase Set I) #7405

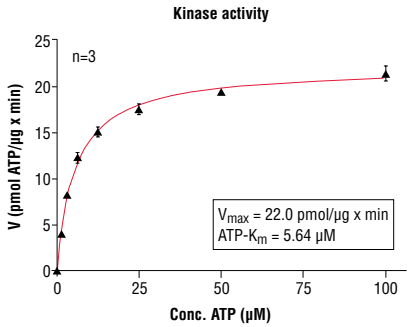

Figure 2. FLT3 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20,000, Substrate: PolyEY, 1.5 µg/50 µl and 200 ng/50 µl Recombinant FLT3.

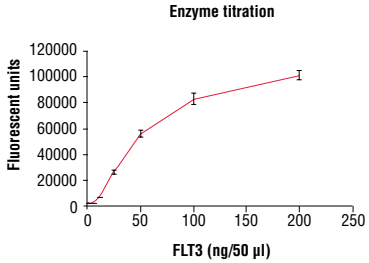

Figure 3. Dose dependence curve of FLT3 kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide (#1310) by FLT3 kinase. In a 50 µl reaction, increasing amounts of FLT3 and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for FLT3 Kinase Assay

***IMPORTANT:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

Kinase

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. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8
4. Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411
5. HTScan™ Tyrosine Kinase Buffer (4X) #9805
6. ATP (10 mM) #9804
7. Gastrin Precursor (Tyr87) Biotinylated Peptide #1310
8. DTT (1000X, 1.25 M)
9. DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
10. DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
11. DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

DELFIA® is a registered trademark of PerkinElmer Life Sciences

B Suggested Protocol for 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with dH_20 to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate] = 3 µm).
2. Immediately transfer enzyme from –80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 10 µl of DTT (1.25 M) to 2.5 ml of 4X HTScan™ Tyrosine Kinase Buffer (240 mM HEPES pH 7.5, 20 mM $MgCl_2$, 20 mM $MnCl_2$, 12 µM Na_3V0_4) to make DTT/Kinase buffer.
5. Transfer 1.25 ml of DTT/Kinase buffer to enzyme tube to make 4X reaction cocktail ([enzyme]=4.0 ng/µl in 4X reaction cocktail).
6. Incubate 12.5 µl of the 4X reaction cocktail with 12.5 µl/well of prediluted compound of interest (usually around 10 µM) for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

Final Assay Conditions for a 50 µl Reaction

60 mM HEPES pH 7.5
5 mM $MgCl_2$
5 mM $MnCl_2$
3 µM Na_3VO_4
1.25 mM DTT
200 µM ATP
1.5 µM peptide
50 ng FLT3 Kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 µl of each reaction and 75 µl of dH_20/well to a 96-well streptavidin-coated plate and incubate at room temperature for 60 minutes.
11. *Wash three times with 200 µl/well PBS/T.
12. Dilute primary antibody in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
    Please note: This protocol was validated using a Gastrin Precursor (Tyr87) Biotinylated Peptide and Phospho-Tyrosine Mouse mAb (P-Tyr-100) diluted 1:1000 (see additional reagents). Primary antibody chosen should be specific to the substrate used.
13. Incubate at room temperature for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. Dilute Europium labeled anti-mouse IgG 1:500 in PBS/T with 1% BSA. Add 100 µl/well diluted antibody.
16. Incubate at room temperature for 30 minutes.
17. *Wash five times with 200 µl/well PBS/T.
18. Add 100 µl/well DELFIA® Enhancement Solution.
19. Incubate at room temperature for 5 minutes.
20. Detect 615 nm fluorescence emission with appropriate Time-Resolved Plate Reader.

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.
Email: drugdiscovery@cellsignal.com