#12596 Store at -20°C

Phospho-FLT3 Antibody Sampler Kit

☑ 1 Kit
(5 x 40 µl)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-FLT3 (Tyr589/591) (30D4) Rabbit mAb	3464	40 µl	160 kDa	Rabbit IgG
Phospho-FLT3 (Tyr591) (33G6) Rabbit mAb	3474	40 µl	160 kDa	Rabbit IgG
Phospho-FLT3 (Tyr842) (10A8) Rabbit mAb	4577	40 µl	160 kDa	Rabbit IgG
Phospho-FLT3 (Tyr969) (C24D9) Rabbit mAb	3463	40 µl	160 kDa	Rabbit IgG
FLT3 (8F2) Rabbit mAb	3462	40 µl	130-160 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Phospho-FLT3 Antibody Sampler Kit provides an economical means of evaluating the FLT3 tyrosine kinase and several phosphorylation sites that are involved in its activation. The kit includes enough antibody to perform four western blot experiments with each primary antibody.

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).

Specificity/Sensitivity: The phospho-FLT3 antibodies recognize the phosphorylated form of FLT3 at the indicated sites. The Phospho-FLT3 (Tyr591) (33G6) Rabbit mAb and Phospho-FLT3 (Tyr969) (C24D9) Rabbit mAb may cross-react with other tyrosine-phosphorylated proteins. The Phospho-FLT3 (Tyr842) (10A8) Rabbit mAb may cross-react with other tyrosine phosphorylated family members. The control FLT3 antibody recognizes both the phosphorylated and nonphosphorylated forms of this kinase.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Tyr589/591, Tyr591, Tyr842 or Tyr969 of human FLT3. The FLT3 antibody is produced by immunizing animals with a synthetic peptide surrounding Ser740 of human FLT3.

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: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

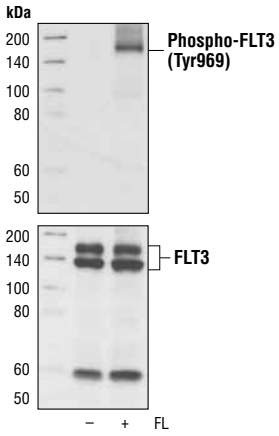
Recommended Antibody Dilutions:

Western blotting	1:1000

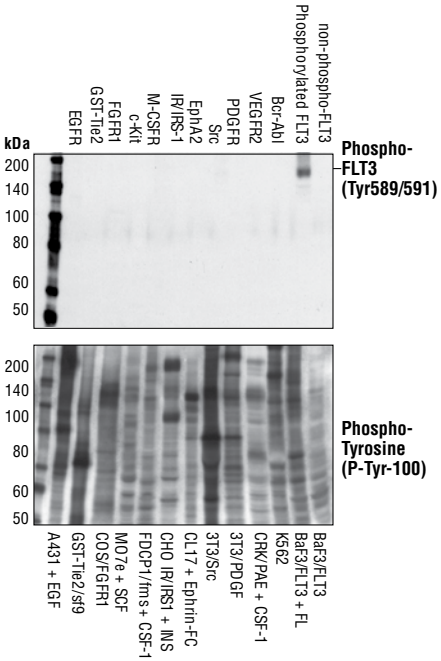
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

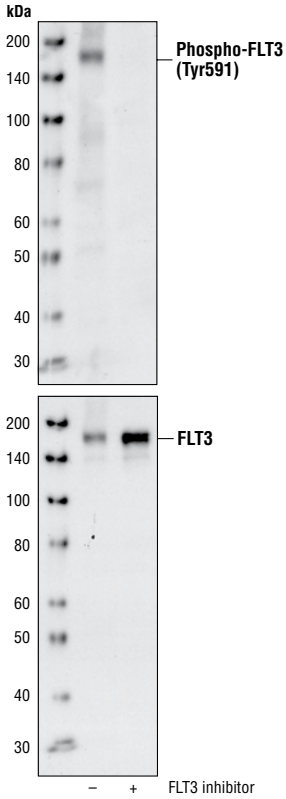
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



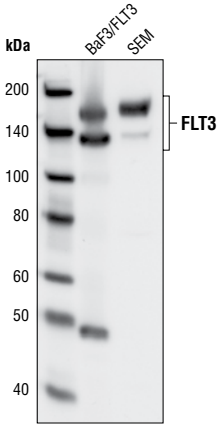
*Western blot analysis of cell extracts from Baf3/FLT3 cells, untreated (-) or treated with FLT3 ligand (FL; +), using **Phospho-FLT3 (Tyr969) (C24D9) Rabbit mAb #3463** (upper) or FLT3 (8F2) Rabbit mAb Antibody #3462 (lower).*



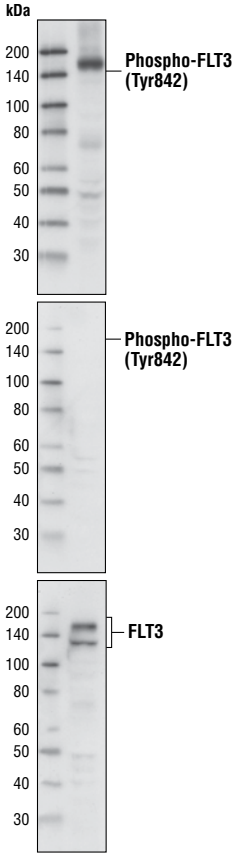
*Western blot analysis of extracts from cells expressing various activated tyrosine kinase proteins using **Phospho-FLT3 (Tyr589/591) (30D4) Rabbit mAb #3464** (upper) or Phospho-Tyrosine mAb (P-Tyr-100) #9411 (lower). Phospho-FLT3 (Tyr589/591) (30D4) Rabbit mAb specifically recognizes the activated FLT3 but not non-phosphorylated FLT3 protein.*



*Western blot analysis of SEM cell lysates, untreated (-) or treated with FLT3 inhibitor (+), using **Phospho-FLT3 (Tyr591) Rabbit mAb #3474** (upper) and FLT3 (8F2) Rabbit mAb #3462 (lower).*



*Western blot analysis of extracts from Baf3/FLT3 transfected cells and SEM leukemia cells using **FLT3 (8F2) Rabbit mAb #3462**.*



*Western blot analysis of cell extracts from SEM cells expressing constitutively activated FLT3 using **Phospho-FLT3 (Tyr842) (10A8) Rabbit mAb #4577** (upper and middle). The middle blot was treated with calf intestinal phosphatase (CIP) before antibody probing. Total FLT3 (8F2) Rabbit mAb #3462 shown at the bottom.*

#12596

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading budder. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X Transfer Buffer: add 100 ml 10X Transfer Buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X Peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.