

#3466 Store at -20°C

Phospho-FLT3 (Tyr591) (54H1) Mouse mAb

100 µl
 (10 Western mini-blots)



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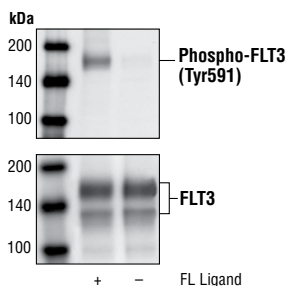
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source	Isotype
W Transfected	H, M	130 kDa nonglycosylated form 160 kDa glycosylated mature form	Mouse	IgG2b

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: Phospho-FLT3 (Tyr591) (54H1) Mouse mAb detects transfected levels of FLT3 only when phosphorylated at tyrosine 591. The antibody may cross-react with some tyrosine-phosphorylated proteins.

Source/Purification: Monoclonal antibody is produced by immunizing mice with a synthetic phospho-peptide (KLH-coupled) corresponding to the sequences surrounding Tyr591 of human FLT3.



Western blot analysis of extracts from Baf3/FLT3 cells, untreated or stimulated with FLT-ligand (3 µg/ml for 2 minutes), using Phospho-FLT3 (Tyr591) (54H1) Mouse mAb (upper) or FLT3 antibody lower.

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.

Entrez-Gene ID # 2322
Swiss-Prot Acc. # P36888

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 antibody.

*Species cross-reactivity is determined by Western blot.

Recommended Antibody Dilutions:
 Western Blotting 1:1000

- Companion Products:**
- Phospho-FLT3 (Tyr591) Antibody #3461
 - FLT3 (8F2) Rabbit mAb #3462
 - Phospho-FLT3 (Tyr589/591) (30D4) Rabbit mAb #3464
 - Phospho-FLT3 (Tyr591) (33G6) Rabbit mAb #3474
 - Phospho-FLT3 (Tyr591) Antibody (Alexa Fluor® 488 Conjugate) #3459
 - Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
 - Anti-rabbit IgG, HRP-linked Antibody #7074
 - Prestained Protein Marker, Broad Range (Premixed Format) #7720
 - Biotinylated Protein Ladder Detection Pack #7727
 - 20X LumiGLO® Reagent and 20X Peroxide #7003

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

F—Flow cytometry E—ELISA D—DELFIATM
 Z—zebra fish B—bovine All—all species expected

Western Immunoblotting Protocol (Primary Antibody Incubation in Milk)

For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7072:** Includes biotinylated protein ladder, secondary anti-mouse (#7076) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight marker (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.