

Phospho-FLT3 (Tyr591) Antibody (Alexa Fluor® 488 Conjugate)

✓ 500 µl
(50 tests)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Applications	Species Cross-Reactivity	Source	Isotype
F	H, M*	Rabbit	IgG

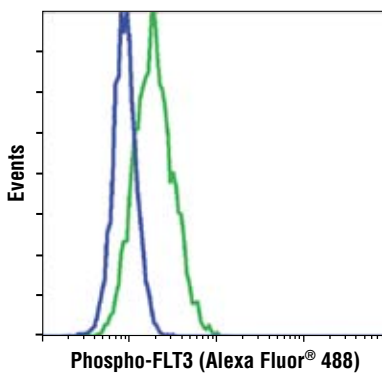
Background: FMS-related tyrosine kinase 3 (FLT3, also called Flk2), is a member of the type III receptor tyrosine kinase family, which includes c-Kit, PDGFR and M-CSF receptors. FLT3 is expressed on early hematopoietic progenitor cells and supports growth and differentiation within the hematopoietic 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).

Description: This Cell Signaling Technology Antibody was conjugated to Alexa Fluor®488 fluorescent dye and tested in-house for direct flow cytometric analysis of human cells. The unconjugated antibody #3461 reacts with human and mouse Phospho-FLT3 (Tyr591). CST expects that Phospho-FLT3 (Tyr591) Antibody (Alexa Fluor® 488 Conjugate) will also recognize Phospho-FLT3 (Tyr591) in these species*.

Directions for Use: Add 10 µl of the conjugated antibody to 500,000 cells in 90 µl PBS/0.5% BSA. See protocol for more details.

Specificity/Sensitivity: Phospho-FLT3 (Tyr591) Antibody detects overexpressed FLT3 only when phosphorylated at tyrosine 591. The antibody does not cross-react with other FLT family members. It may cross-react with some tyrosine phosphorylated proteins. (Patent Pending.)

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Tyr591 of human FLT3. Antibodies are purified by protein A and peptide affinity chromatography. The antibody was conjugated to Alexa Fluor® 488 under optimal conditions with an F/P ratio of 2-5.



Flow cytometric analysis of SEM cells, treated with a FLT3 inhibitor (blue) or untreated (green), using Phospho-FLT3 (Tyr591) Antibody (Alexa Fluor® 488 Conjugate).

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 PBS (pH 7.2), 0.1% Sodium azide, 2 mg/ml BSA. Store at 4°C. *Protect from light. Do not freeze.*

Recommended Antibody Dilutions:

Flow Cytometry 1:10

Companion Products:

Phospho-FLT3 (Tyr591) Antibody #3461
Phospho-FLT3 (Tyr591) (54H1) Mouse mAb #3466
Phospho-FLT3 (Tyr589/591) (30D4) Rabbit mAb #3464
FLT3 Kinase #7742

The Alexa Fluor® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc., for research use only, except for use in combination with DNA microarrays. The Alexa Fluor® dyes (except for Alexa Fluor® 430 dye) are covered by pending and issued patents.

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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—zebra fish B—bovine
Dg—Dog Pg—Pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Flow Cytometry Protocol for Intracellular Staining Using Conjugated Primary Antibodies

A Solutions and Reagents

1. **1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 800 ml distilled water (dH_2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
2. Formaldehyde (methanol free)
3. **Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100ml 1X PBS. Store at 4°C

B Fixation

1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
3. Fix for 10 minutes at 37°C.
4. Chill tubes on ice for 1 minute.

C Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 minutes on ice.
3. Proceed with staining or store cells at -20°C in 90% methanol.

D Staining Using Conjugated Primary Antibodies

NOTE: Allow for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemacytometer or alternative method.

1. Aliquot 5×10^5 cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation.
3. Resuspend cells in 90 μl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add 10 μl of conjugated antibody to the assay tubes.
6. Incubate for 30-60 minutes, in the dark at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.