Store at -20°C

#9370

c-Kit Antibody Sampler Kit

☑ 1 Kit
(3 x 20 µl)

Cell Signaling TECHNOLOGY®

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rev. 06/16

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-c-Kit (Tyr703) (D12E12) Rabbit mAb	3073	20 µl	145 kDa	Rabbit IgG
Phospho-c-Kit (Tyr719) Antibody	3391	20 µl	120, 145 kDa	Rabbit IgG
c-Kit (D13A2) XP™ Rabbit mAb	3074	20 µl	120, 145 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.

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 validation data and a complete listing of recommended companion products.

Description: The c-Kit Antibody Sampler Kit provides a fast and economical means of evaluating levels of c-Kit receptor protein phosphorylated at the specified sites, as well as total c-Kit receptor levels. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: c-Kit is a member of the subfamily of receptor tyrosine kinases that includes PDGF, CSF-1 and FLT3/flk-2 receptors (1,2). It plays a critical role in activation and growth in a number of cell types including hematopoietic stem cells, mast cells, melanocytes and germ cells (3). Upon binding with its stem cell factor (SCF) ligand, c-Kit undergoes dimerization/oligomerization and autophosphorylation. Activation of c-Kit results in the recruitment and tyrosine phosphorylation of downstream SH2-containing signaling components including PLCγ, the p85 subunit of PI3 kinase, SHP2 and CrkL (4). Molecular lesions that impair the kinase activity of c-Kit are associated with a variety of developmental disorders (5), while mutations that constitutively activate c-Kit can lead to pathogenesis of mastocytosis and gastrointestinal stromal tumors (6). Tyr719 is located in the kinase insert region of the catalytic domain. c-Kit phosphorylated at Tyr719 binds to the p85 subunit of PI3 kinase *in vitro* and *in vivo* (7).

Specificity/Sensitivity: Phospho-c-Kit antibodies detect endogenous levels of c-Kit when phosphorylated at the specified sites. c-Kit XP™ (D13A2) Rabbit mAb detects endogenous levels of total c-kit protein.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide (KLH-coupled) corresponding to the residues surrounding Tyr703 of human c-Kit. Polyclonal antibody is produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Tyr719 of mouse c-Kit. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Martin, F.H. et al. (1990) *Cell* 63, 203-11.

(2) Yarden, Y. et al. (1987) *EMBO J* 6, 3341-51.

(3) Gommerman, J.L. et al. (1997) *J Biol Chem* 272, 30519-25.

(4) Sattler, M. et al. (1997) *J Biol Chem* 272, 10248-53.

(5) Nocka, K. et al. (1990) *EMBO J* 9, 1805-13.

(6) Hirota, S. et al. (1998) *Science* 279, 577-80.

(7) Blume-Jensen, P. et al. (2000) *Nat Genet* 24, 157-62.

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.

#9370

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 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.

1. 1X Phosphate Buffered Saline (PBS)
2. **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
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **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).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **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%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope®-HRP Western Blot Detection System** #7071**:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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.

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 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

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

8. Electrotransfer to nitrocellulose or PVDF membrane.

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.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. 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.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. 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.

2. 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.