Store at -20°C

#9779

Pim Kinase Antibody Sampler Kit

☑ 1 Kit
(5 x 20 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
Pim-1 (C93F2) Rabbit mAb	3247	20 µl	34 kDa	Rabbit IgG
Pim-2 (D1D2) XP™ Rabbit mAb	4730	20 µl	34, 38, 40 kDa	Rabbit IgG
Pim-3 (D17C9) Rabbit mAb	4165	20 µl	35 kDa	Rabbit IgG
Phospho-Bad (Ser112) (40A9) Rabbit mAb	5284	20 µl	23 kDa	Rabbit IgG
Bad (D24A9) Rabbit mAb	9239	20 µl	23 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 Pim Kinase Antibody Sampler Kit provides an economical means to detect all three Pim kinases along with Bad and Phospho-Bad (Ser112). The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Pim proteins (Pim-1, Pim-2 and Pim-3) are oncogene-encoded serine/threonine kinases (1). Pim-1, a serine/threonine kinase highly expressed in hematopoietic cells, plays a critical role in the transduction of mitogenic signals and is rapidly induced by a variety of growth factors and cytokines (1-4). Pim-1 cooperates with c-Myc in lymphoid cell transformation and protects cells from growth factor withdrawal and genotoxic stress-induced apoptosis (5,6). Pim-1 also enhances the transcriptional activity of c-Myb through direct phosphorylation within the c-Myb DNA binding domain as well as phosphorylation of the transcriptional coactivator p100 (7,8). Hypermutations of the Pim-1 gene are found in B-cell diffuse large cell lymphomas (9). Phosphorylation of Pim-1 at Tyr218 by Etk occurs following IL-6 stimulation and correlates with an increase in Pim-1 activity (10). Various Pim substrates have been identified; Bad is phosphorylated by both Pim-1 and Pim-2 at Ser112 and this phosphorylation reverses Bad-induced cell apoptosis (11,12). The corresponding pim-1 gene encodes a pair of proteins through use of different translation initiation sites. Both larger 44 kDa (Pim-1L) and smaller 33 kDa (Pim-1S) proteins are active kinases, but differ in stability (13).

Specificity/Sensitivity: The Pim-1 (C93F2) Rabbit mAb, Pim-2 (D1D2) XP™ Rabbit mAb, and Pim-3 (D17C9) Rabbit mAb detect endogenous levels of the respective proteins. The Pim antibodies do not crossreact with other Pim family members. Phospho-Bad (Ser112) (40A9) Rabbit mAb detects endogenous levels of Bad only when phosphorylated at Ser112. It does not detect Bad phosphorylated at other sites, nor does it detect related family members. Bad (D24A9) Rabbit mAb detects endogenous levels of total Bad protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Val160 of human Pim-1, Cys266 of human Pim-2, Ser275 of human Pim-3, Ser112 of mouse Bad, and Pro102 of human Bad, respectively.

Background References:

(1) Mikkers, H. et al. (2004) *Mol Cell Biol* 24, 6104-15.
(2) Selten, G. et al. (1986) *Cell* 46, 603-11.
(3) Meeker, T.C. et al. (1987) *J Cell Biochem* 35, 105-12.
(4) Dautry, F. et al. (1988) *J Biol Chem* 263, 17615-20.
(5) Möröy, T. et al. (1993) *Proc Natl Acad Sci USA* 90, 10734-8.
(6) Lilly, M. and Kraft, A. (1997) *Cancer Res* 57, 5348-55.
(7) Leverson, J.D. et al. (1998) *Mol Cell* 2, 417-25.
(8) Winn, L.M. et al. (2003) *Cell Cycle* 2, 258-62.
(9) Pasqualucci, L. et al. (2001) *Nature* 412, 341-6.
(10) Kim, O. et al. (2004) *Oncogene* 23, 1838-44.
(11) Aho, T.L. et al. (2004) *FEBS Lett* 571, 43-9.
(12) Yan, B. et al. (2003) *J Biol Chem* 278, 45358-67.
(13) Saris, C.J. et al. (1991) *EMBO J* 10, 655-64.

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 **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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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.