

#4723 Store at -20°C

# Pim-2 Antibody

100 µl  
 (10 Western mini-blot)

**Orders** ■ 877-616-CELL (2355)  
 orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
 info@cellsignal.com  
**Web** ■ www.cellsignal.com

New 05/07

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

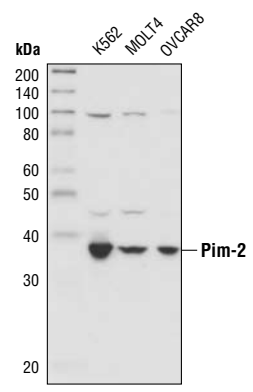
Applications	Species Cross-Reactivity	Molecular Wt.	Source
W	H, (Mk)	34, 38, 40 kDa	Rabbit

**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 is correlated 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).

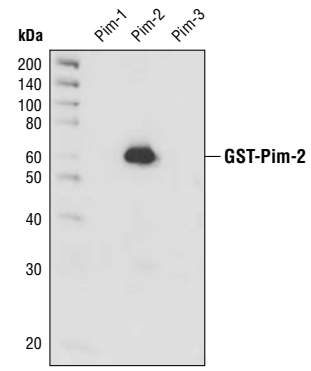
Pim-2 is highly homologous to Pim-1 with similar oncogenic functions (13,14). Three isoforms of Pim-2 can be generated from alternative start sites which run at 34, 38, and 40 kDa (13). Pim-2 leads to resistance to a variety of apoptotic stimuli and its expression is negatively regulated by growth factor withdrawal (15,16). Increased levels of Pim-2 has also been observed in certain cancers (17,18).

**Specificity/Sensitivity:** Pim-2 Antibody detects endogenous levels of total Pim-2 protein. The antibody does not cross-react with other Pim family members.

**Source/Purification:** Polyclonal antibodies were prepared by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues surrounding Gln287 of human Pim-2. Antibodies were purified by peptide affinity chromatography.



Western blot analysis of extracts from K562, MOLT4 and OVCAR8 cell lines using Pim-2 Antibody.



Western blot analysis of recombinant Pim-1, Pim-2 and Pim-3 kinases using Pim-2 Antibody.

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

**Recommended Antibody Dilutions:**  
 Western blotting 1:1000

**Companion Products:**  
 Phospho-Pim-1 (Tyr218) Antibody #4721  
 Pim-1 Antibody #4722  
 Pim-1 Kinase #7572  
 Pim-2 Kinase #7575  
 HTScan® Pim-1 Kinase Assay Kit #7573  
 HTScan® Pim-2 Kinase Assay Kit #7576

**Background References:**  
 (1) Mikkers, H. et al. (2004) *Mol. Cell.Biol.* 24, 6104-6115.  
 (2) Seltzen, G. et al. (1986) *Cell* 46, 603-611.  
 (3) Meeker, T.C. et al. (1987) *J. Cell. Biochem.* 35, 105-112.  
 (4) Dautry, F. et al. (1988) *J. Biol. Chem.* 263, 17615-17620.  
 (5) Moroy, T. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90, 10734-10738.  
 (6) Lilly, M. and Kraft, A. (1997) *Cancer Res.* 57, 5348-5355.  
 (7) Levenson, J.D. et al. (1998) *Mol. Cell* 2, 417-425.  
 (8) Winn, L.M. et al. (2003) *Cell Cycle* 2, 258-262.  
 (9) Pasqualucci, L. et al. (2001) *Nature* 412, 341-346.  
 (10) Kim, O. et al. (2004) *Oncogene* 23, 1838-1844.  
 (11) Aho, T.L. et al. (2004) *FEBS Lett.* 571, 43-49.  
 (12) Yan, B. et al. (2003) *J. Biol. Chem.* 278, 45358-45367.  
 (13) van der Lugt, N.M. et al. (1995) *EMBO J.* 14, 2536-2544.  
 (14) Breuer, M.L. et al. (1989) *EMBO J.* 8, 743-748.  
 (15) Fox, C.J. et al. (2003) *Genes Dev.* 17, 1841-1854.  
 (16) White, E. (2003) *Genes Dev.* 17, 1813-1816.  
 (17) Cohen, A.M. et al. (2004) *Leuk. Lymphoma* 45, 951-955.  
 (18) Dai, H. et al. (2005) *Prostate* 65, 276-286.

**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  
 F—Flow cytometry E—ELISA D—DELFIATM  
 Z—zebra fish B—bovine All—all species expected  
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

## Western Immunoblotting Protocol (Primary Ab 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)
- Bovine Serum Albumin (BSA)
- 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 #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.
- 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 markers (#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 cm<sup>2</sup>) 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.