

Phospho-Bim (Ser77) (D4H12) Rabbit mAb



✓ 100 µl
 (10 western blots)

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New 04/13

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

Entrez-Gene ID #10018
 Swiss-Prot Acc. #043521

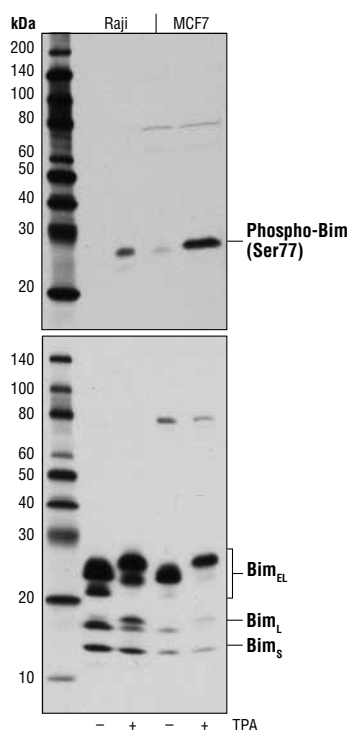
Applications W, IP Endogenous	Species Cross-Reactivity* H, M, (Mk)	Molecular Wt. 26 kDa	Isotype Rabbit IgG**
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Background: Bim/Bod is a pro-apoptotic protein belonging to the BH3-only group of Bcl-2 family members including Bad, Bid, Bik, Hrk, and Noxa that contain a BH3 domain but lack other conserved BH1 or BH2 domains (1,2). Bim induces apoptosis by binding to and antagonizing anti-apoptotic members of the Bcl-2 family. Interactions have been observed with Bcl-2, Bcl-xL, Mcl-1, Bcl-w, Bfl-1, and BHRF-1 (1,2). Bim functions in regulating apoptosis associated with thymocyte negative selection and following growth factor withdrawal, during which Bim expression is elevated (3-6). Three major isoforms of Bim are generated by alternative splicing: Bim_L, Bim_M, and Bim_S (1). The shortest form, Bim_S, is the most cytotoxic and is generally only transiently expressed during apoptosis. The Bim_L and Bim_M isoforms may be sequestered to the dynein motor complex through an interaction with the dynein light chain and released from this complex during apoptosis (7). Apoptotic activity of these longer isoforms may be regulated by phosphorylation (8,9). Environmental stress triggers Bim phosphorylation by JNK and results in its dissociation from the dynein complex and increased apoptotic activity.

MAP kinase dependent phosphorylation of Bim at multiple sites, including Ser55, Ser65, and Ser73 in mouse (Ser59, Ser69, and Ser77 in human), can promote proteasomal degradation of Bim and inhibition of apoptosis (10).

Specificity/Sensitivity: Phospho-Bim (Ser77) (D4H12) Rabbit mAb recognizes endogenous levels of Bim protein only when phosphorylated at Ser77.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser77 of human Bim protein.



Western blot analysis of extracts from Raji and MCF7 cells, untreated (-) or treated with TPA #4174 (200 nM, 30 min; +), using Phospho-Bim (Ser77) (D4H12) Rabbit mAb (upper) or Bim (C34C5) Rabbit mAb #2933 (lower).

Immunoprecipitation of Phospho-Bim (Ser77) from extracts of MCF7 cells treated with TPA #4174 (200 nM, 30 min), using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or Phospho-Bim (Ser77) (D4H12) Rabbit mAb (lane 3). Lane 1 is 10% input. Mouse Anti-rabbit IgG (Conformation Specific) (L27A9) mAb (HRP Conjugate) #5127 was used to avoid recognition of rabbit IgG heavy and light chains by western blot.

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.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

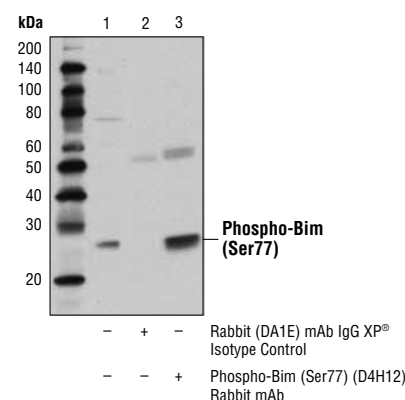
Western blotting 1:1000
 Immunoprecipitation 1:100

For product specific protocols please see the web page for this product at www.cellsignaling.com.

Please visit www.cellsignaling.com for a complete listing of recommended complementary products.

Background References:

- (1) O'Connor, L. et al. (1998) *EMBO J* 17, 384-95.
- (2) Hsu, S.Y. et al. (1998) *Mol Endocrinol* 12, 1432-40.
- (3) Bouillet, P. et al. (2002) *Nature* 415, 922-6.
- (4) Whitfield, J. et al. (2001) *Neuron* 29, 629-43.
- (5) Dijkers, P.F. et al. (2000) *Curr Biol* 10, 1201-4.
- (6) Ley, R. et al. (2003) *J Biol Chem* 278, 18811-6.
- (7) Puthalakath, H. et al. (1999) *Mol Cell* 3, 287-96.
- (8) Lei, K. and Davis, R.J. (2003) *Proc Natl Acad Sci USA* 100, 2432-7.
- (9) Putcha, G.V. et al. (2003) *Neuron* 38, 899-914.
- (10) Hübner, A. et al. (2008) *Mol Cell* 30, 415-25.



IMPORTANT: 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.

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.