

#9291 Store at **-20°C**

Phospho-Bad (Ser112) Antibody

- Small 100 μ l (10 Western mini-blot)
- Large 300 μ l (30 Western mini-blot)



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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* | Molecular Wt. | Source |
|-----------------------------|---------------------------|---------------|----------|
| W, IP, E-P, F Endogenous | H, M, R, Mk | 23 kDa | Rabbit** |

Background: Bad is a proapoptotic member of the Bcl-2 family that can displace Bax from binding to Bcl-2 and Bcl-xL, resulting in cell death (1,2). Survival factors such as IL-3 can inhibit the apoptotic activity of Bad by activating intracellular signaling pathways that result in the phosphorylation of Bad at Ser112 and Ser136 (2). Phosphorylation at these sites results in the binding of Bad to 14-3-3 proteins and the inhibition of Bad binding to Bcl-2 and Bcl-xL (2). Akt has been shown to promote cell survival via its ability to phosphorylate Bad at Ser136 (3,4). Bad is phosphorylated at Ser112 both *in vivo* and *in vitro* by p90RSK (5,6) and mitochondria-anchored PKA (7). Phosphorylation of Ser155 in the BH3 domain by PKA plays a critical role in blocking the dimerization of Bad and Bcl-xL (8-10).

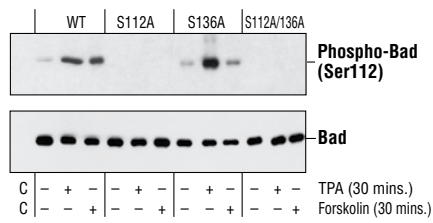
Specificity/Sensitivity: Phospho-Bad (Ser112) Antibody detects endogenous levels of Bad only when phosphorylated at Ser112. This antibody does not detect Bad phosphorylated at other sites, nor does it detect related family members.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phospho-peptide corresponding to residues surrounding Ser112 of mouse Bad. Antibodies are purified by protein A and peptide affinity chromatography.

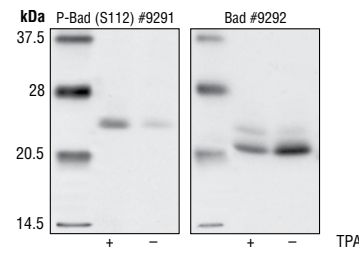
Selected Application References:
 Jamieson, C.A. and Yamamoto, K.R. (2000) Crosstalk pathway for inhibition of glucocorticoid-induced apoptosis by T cell receptor signaling. *Proc. Natl. Acad. Sci. USA* 97, 7319-7324. Application: W.

Tan, Y. et al. (1999) p90(RSK) blocks bad-mediated cell death via a protein kinase C-dependent pathway. *J. Biol. Chem.* 274, 34859-34867. Application: W.

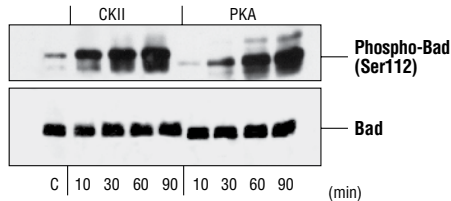
Bertolotto, C. et al. (2000) Protein kinase C τ and ϵ promote T-cell survival by a Rsk-dependent phosphorylation and inactivation of BAD. *J. Biol. Chem.* 275 (47), 37246-37250. Application: W.



Western blot analysis of extracts from 293 cells transfected with Wild-type Bad, Bad (Ser112A), Bad (S136A), Bad (S112A/S136A) and treated with TPA or forskolin, using Phospho-Bad (Ser112) Antibody (upper) or Bad Antibody #9292 (lower).



Western blot analysis of extracts from COS cells, untreated or TPA-treated, using Bad Antibody #9292 (right) or Phospho-Bad (Ser112) Antibody (left).



Western blot analysis of Bad fusion protein phosphorylated by CKII or PKA *in vitro*, using Phospho-Bad (Ser112) Antibody (upper) or Bad Antibody #9292 (lower).

Entrez-Gene ID # 12015
Swiss-Prot Acc. # Q61337

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ g/ml BSA and 50% glycerol. 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 | 1:50 |
| ELISA (Peptide) | 1:1000 |
| Flow Cytometry | 1:100 |

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

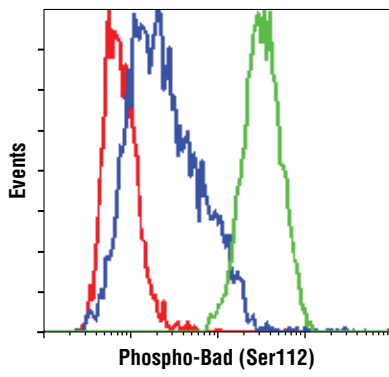
- Companion Products:**
- Phospho-Bad (Ser112) (40A9) Rabbit mAb #5284
 - PhosphoPlus® Bad (Ser112/136) Antibody Kit #9290
 - Bad Antibody #9292
 - Bad Control Proteins #9293
 - Phospho-Bad (Ser136) Antibody #9295

Please visit www.cellsignal.com for a complete listing of recommended companion products.

- Background References:**
- (1) Yang, E. et al. (1995) *Cell* 80, 285-291.
 - (2) Zha, J. et al. (1996) *Cell* 87, 619-628.
 - (3) Datta, S.R. et al. (1997) *Cell* 91, 231-241.
 - (4) Peso, L. et al. (1997) *Science* 278, 687-689.
 - (5) Bonni, A. et al. (1999) *Science* 286, 1358-1362.
 - (6) Tan, Y. et al. (1999) *J. Biol. Chem.* 274, 34859-34867.
 - (7) Harada, H. et al. (1999) *Mol. Cell* 3, 413-422.
 - (8) Tan, Y. et al. (2000) *J. Biol. Chem.* 275, 25865-25869.
 - (9) Lizcano, J. et al. (2000) *Biochem. J.* 349, 547-557.
 - (10) Datta, S. et al. (2000) *Mol. Cell* 6, 41-51.

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—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 cytometric analysis of COS cells, untreated (blue) or Calyculin A/TPA-treated (green), using Phospho-Bad (Ser112) Antibody compared to Rabbit (DA1E) mAb IgG Isotype Control #3900 (red).