



Store at -20°

±9295

Applications	Species Cross-Reactivity*	Source
W Transfected	M, (H, R)	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 (Ser136) Antibody detects transfected levels of Bad only when phosphorylated at Ser136. 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 Ser136 of mouse Bad. Antibodies are purified by protein A and peptide affinity chromatography.

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.



Western blot analysis of GST-Bad, phosphorylated by CKII or PKA in vitro, using Phospho-Bad (Ser112) Antibody #9291 (top), Phospho-Bad (Ser136) Antibody (middle) or Bad Antibody #9292 (bottom).



Western blot analysis of extracts from 293 cells transfected with Wild-type Bad, Bad (S112A), Bad (S136A) or Bad (S112A/ S136A), untreated, TPA-treated or forskolin-treated, using Phospho-Bad (Ser112) Antibody #9291 (top), Phospho-Bad (Ser136) Antibody (middle) or Bad Antibody #9292 (bottom).



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Entrez-Gene ID #572 Swiss-Prot Acc. #Q92934

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

1:500

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

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

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.