

#5284 Store at -20°C

Phospho-BAD (Ser112) (40A9) Rabbit mAb

- Small 100 μl
(10 western blots)
- Large 300 μl
(30 western blots)



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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IHC-P, F Endogenous	H, M, R, Mk	23 kDa	Rabbit IgG**

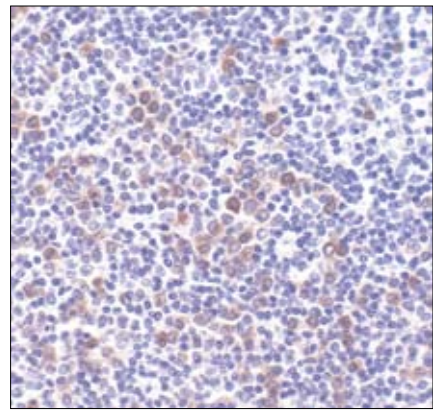
Background: Bad is a proapoptotic member of the Bcl-2 family that can displace Bax from binding to Bcl-2 and Bcl-xL, resulting in cell death (1,2). Survival factors such as IL-3 can inhibit the apoptotic activity of Bad by activating intracellular signaling pathways that result in the phosphorylation 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) (40A9) Rabbit mAb detects endogenous levels of Bad only when phosphorylated at serine 112.

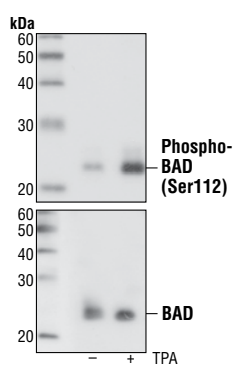
Source/Purification: Phospho-Bad (Ser112) Antibody detects endogenous levels of Bad only when phosphorylated at Ser112. The Ser112 nomenclature is based upon the mouse sequence. The analogous phosphorylation site is Ser75 in human and Ser113 in rat. This antibody does not detect Bad phosphorylated at other sites, nor does it detect related family members.

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



Immunohistochemical analysis of paraffin-embedded Non-Hodgkin's lymphoma, using Phospho-BAD (Ser112) (40A9) Rabbit mAb.



Western blot analysis of extracts from untreated or TPA-treated COS cells, using Phospho-Bad (Ser112) (40A9) Rabbit mAb (upper), or Bad Antibody #9292 (lower).

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

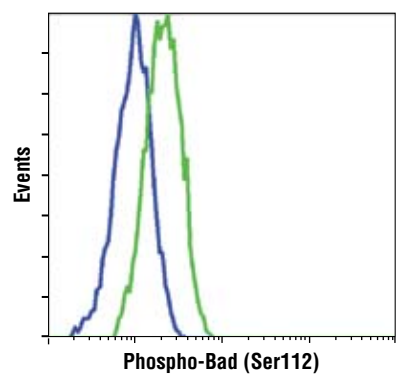
Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 $\mu\text{g}/\text{ml}$ BSA and 50% glycerol. Store at -20°C . Do not aliquot the antibody.

***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
Immunohistochemistry (Paraffin)	1:50
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Flow Cytometry	1:200

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.

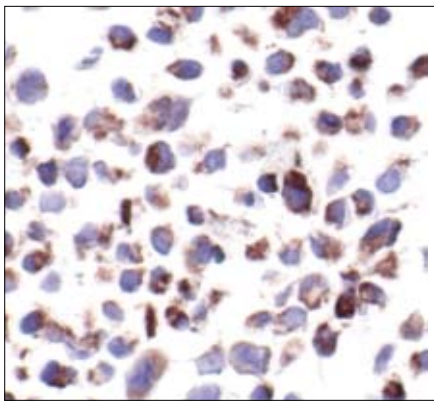
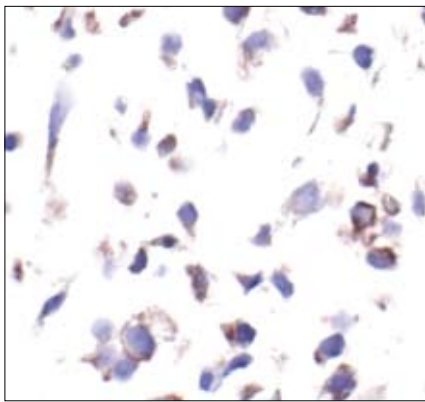


Flow cytometric analysis of COS cells, untreated (blue) or TPA/Calyculin A treated (green), using Phospho-BAD (Ser112) (40A9) Rabbit mAb.

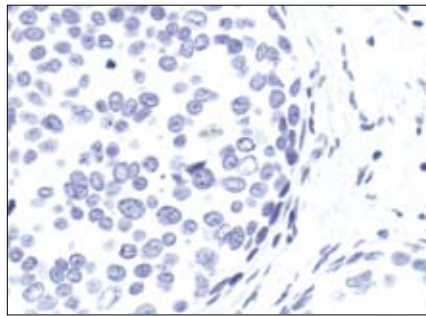
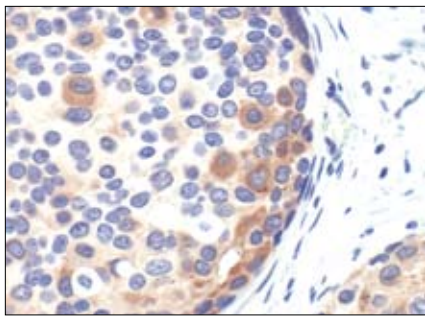
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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.

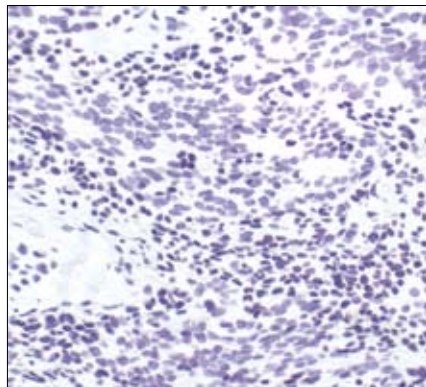
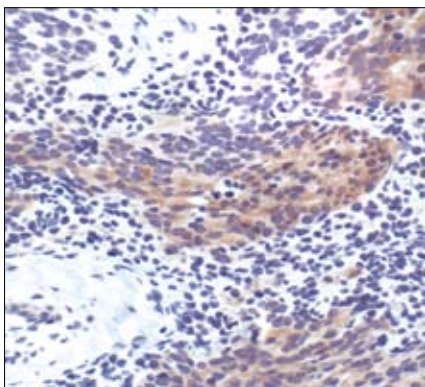
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Immunohistochemical analysis of paraffin embedded COS cells untreated (left) or TPA-treated (right), showing induced cytoplasmic staining, using Phospho-BAD (Ser112) (40A9) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, untreated (left) or λ phosphatase treated (right), using Phospho-Bad (Ser 112) (40A9) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human prostate carcinoma, using Phospho-Bad (Ser 112) (40A9) Rabbit mAb preincubated with control peptide (left) or Phospho-Bad (Ser 112) Blocking Peptide #1026 (right).