

# Phospho-(Ser) 14-3-3 Binding Motif Antibody

100 µl  
 (10 western blots)

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

rev. 05/21/18

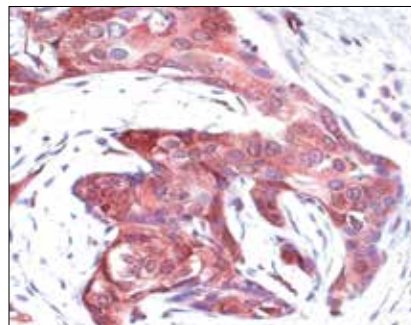
**For Research Use Only. Not For Use In Diagnostic Procedures.**

Applications	Species Cross-Reactivity*	Source**	Motif
W, IP, IHC-P, E-P Endogenous	All	Rabbit	(R/K)XX(S*)XP

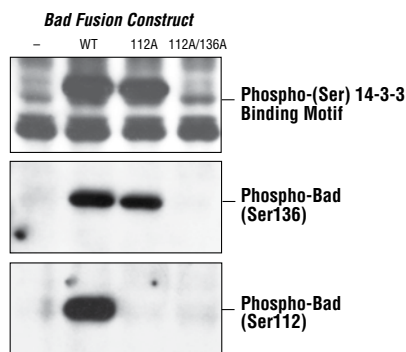
**Background:** The 14-3-3 proteins are a highly conserved family of proteins involved in the regulation of cell survival, apoptosis, proliferation and checkpoint control (1-5). Biological regulation by 14-3-3 is mediated through phosphorylation-dependent protein-protein interactions (6). Two different phospho-Ser-containing motifs are found within nearly all known 14-3-3 binding proteins (7). Motif 1 (Arg/Lys and Ser at positions -3 and -2, phospho-Ser at position 0, and Pro at position +2) is found in critical regulatory proteins including Bad, cdc25C, FKHL1, PKC and c-Raf (5,7). Phospho-(Ser) 14-3-3 Binding Motif Polyclonal and (4E2) Monoclonal Antibodies provide powerful tools for the discovery and characterization of potential 14-3-3 binding proteins containing this motif and for high throughput drug discovery.

**Specificity/Sensitivity:** Phospho-(Ser) 14-3-3 Binding Motif Antibody binds peptides and proteins containing a motif composed of phospho-Ser with proline at the +2 position and arginine or lysine at the -3 position. Antibody binding is phospho-specific and largely independent of other surrounding amino acids. The antibody weakly cross-reacts with sequences containing phospho-Thr instead of phospho-Ser in this motif, and with sequences containing phospho-Ser surrounded by Phe at the +1 position and Arg/Lys at the -3 position. No cross-reactivity is observed with corresponding nonphosphorylated sequences or with other phospho-Thr/Ser/Tyr-containing motifs. By ELISA this antibody recognizes a wide range of peptides containing the 14-3-3 binding motif, and by 2D gel Western blot analysis it recognizes a large number of presumptive 14-3-3 binding proteins. (U.S. Patent No's.: 6,441,140; 6,982,318; 7,259,022; 7,344,714; U.S.S.N. 11,484,485; and all foreign equivalents.)

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with synthetic phospho-(Ser) 14-3-3-binding-motif containing peptides. Antibodies are purified by protein A and peptide affinity chromatography.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, showing staining of proteins containing phosphorylated 14-3-3 binding motifs, using Phospho-(Ser) 14-3-3 Binding Motif Antibody.



Western blot analysis of extracts from 293 cells transfected with Bad or Bad Ser112 and/or Ser136 mutant fusion proteins, using Phospho-(Ser) 14-3-3 Binding Motif Antibody (top), Phospho-Bad (Ser136) Antibody #9295 (middle) or Phospho-Bad (Ser112) Antibody #9291 (bottom).

**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.

**\*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
Immunohistochemistry (Paraffin)	1:50†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114
†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.	
ELISA-Peptide	1:500

**For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).**

**Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.**

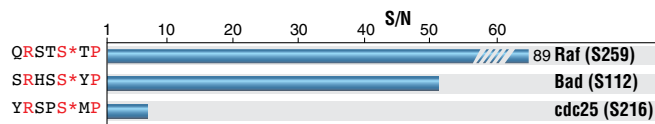
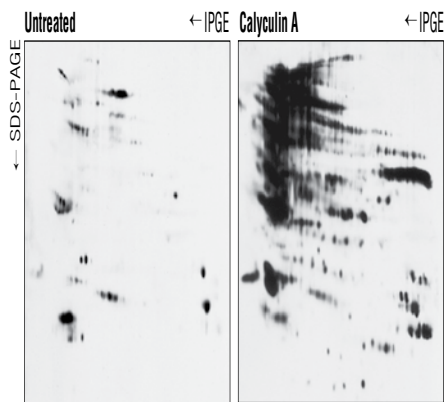
**Background References:**

- (1) Aitken, A. (1995) *Trends Biochem Sci* 20, 95-7.
- (2) Zha, J. et al. (1996) *Cell* 87, 619-28.
- (3) Piwnicka-Worms, H. (1999) *Nature* 401, 535, 537.
- (4) Tzivion, G. et al. (1998) *Nature* 394, 88-92.
- (5) Xing, H. et al. (2000) *EMBO J* 19, 349-58.
- (6) Muslin, A.J. et al. (1996) *Cell* 84, 889-97.
- (7) Yaffe, M.B. et al. (1997) *Cell* 91, 961-71.

**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.**

**License/Use Restrictions:** Use of CST Motif Antibodies within certain methods (e.g., U.S. Patent No.'s 7,198,896 & 7,300,753) may require a license from CST. For information regarding academic licensing terms please have your technology transfer office contact CST Legal Department at CST\_ip@cellsignal.com. For information regarding commercial licensing terms please contact CST Pharma Services Department at ptmscan@cellsignal.com.

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E—ELISA 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.



Phospho-(Ser) 14-3-3 Binding Motif Antibody ELISA Assay: Signal-to-noise ratio of phospho- versus nonphospho-14-3-3 binding motif peptides. (S\* denotes phosphorylated serine.)

Western blot analysis of extracts from Jurkat cells, untreated or calyculin A-treated (0.1  $\mu$ M for 30 minutes), using Phospho-(Ser) 14-3-3 Binding Motif Antibody. Proteins were separated by 2-D electrophoresis prior to blotting.