

#9198 Store at -20°C

Phospho-CREB (Ser133) (87G3) Rabbit mAb

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



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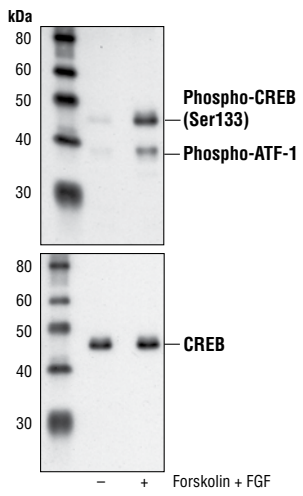
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IHC-P, IHC-F, IF-F, IF-IC, ChIP, F Endogenous	H, M, R	43 kDa	Rabbit IgG**

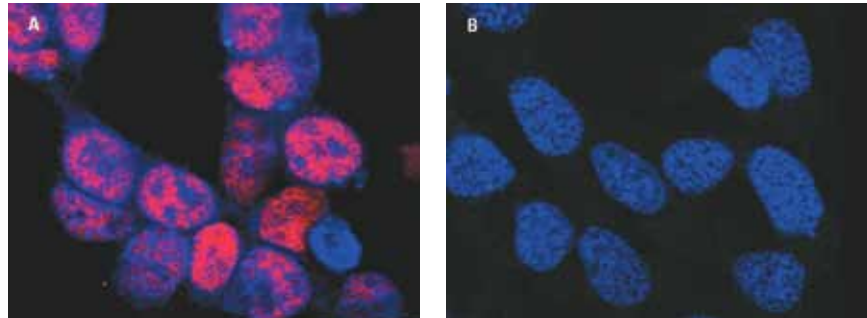
Background: CREB is a bZIP transcription factor that activates target genes through cAMP response elements. CREB is able to mediate signals from numerous physiological stimuli, resulting in regulation of a broad array of cellular responses. While CREB is expressed in numerous tissues, it plays a large regulatory role in the nervous system. CREB is believed to play a key role in promoting neuronal survival, precursor proliferation, neurite outgrowth and neuronal differentiation in certain neuronal populations (1–3). Additionally, CREB signaling is involved in learning and memory in several organisms (4–6). CREB is able to selectively activate numerous downstream genes through interactions with different dimerization partners. CREB is activated by phosphorylation at Ser133 by various signaling pathways including Erk, Ca^{2+} and stress signaling. Some of the kinases involved in phosphorylating CREB at Ser133 are p90RSK, MSK, CaMKIV and MAPKAPK-2 (7–9).

Specificity/Sensitivity: Phospho-CREB (Ser133) (87G3) Rabbit mAb detects endogenous levels of CREB only when phosphorylated at serine 133. The antibody also detects the phosphorylated form of the CREB-related protein, ATF-1.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser133 of human CREB.



Western blot analysis of extracts from SK-N-MC cells, untreated or forskolin- and FGF-treated, using Phospho-CREB (Ser133) (87G3) Rabbit mAb (upper) or CREB (48H2) Rabbit mAb #9197 (lower).



Confocal microscopic images of SK-N-MC cells showing nuclear staining after 25 minute treatment with Forskolin and IBMX using Phospho-CREB (Ser133) (87G3) Rabbit mAb (left, red) compared to untreated cells (right). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

Entrez-Gene ID #1385
Swiss-Prot Acc. #P16220

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 $\mu\text{g}/\text{ml}$ BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C . Do not aliquot the antibody.

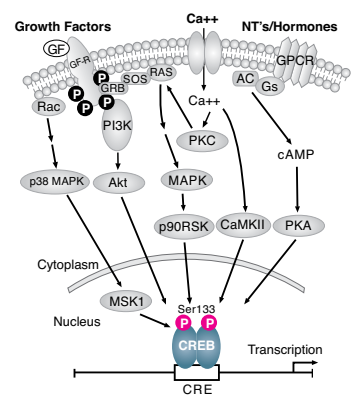
***Species cross-reactivity is determined by western blot.**
****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunohistochemistry (Paraffin)	1:800†
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.	
Immunohistochemistry (Frozen)	1:400
Fixative	10% Neutral buffered formalin
Immunofluorescence (IF-IC)	1:800
Immunofluorescence (IF-F)	1:800
Chromatin IP	1:50
Flow Cytometry	1:800

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.

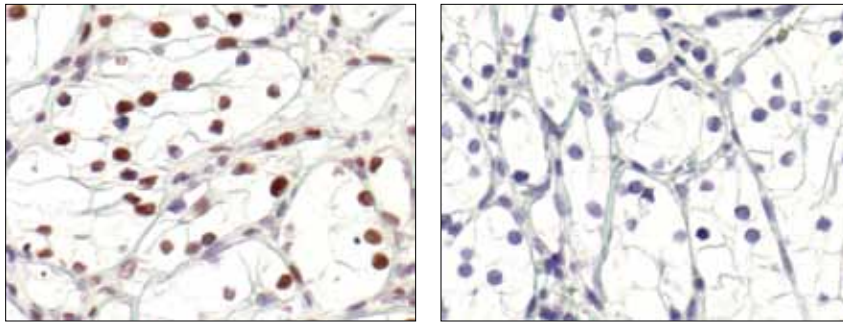


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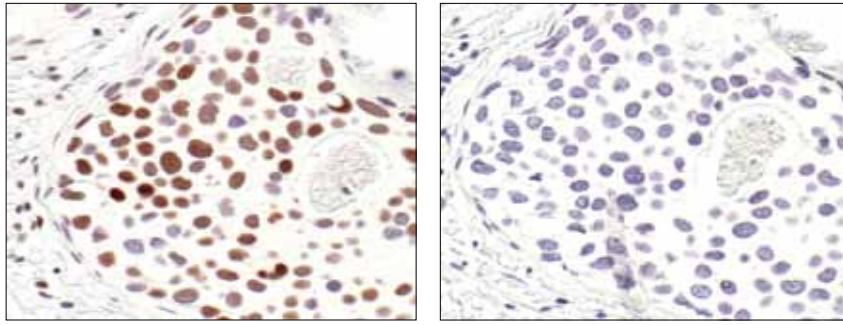
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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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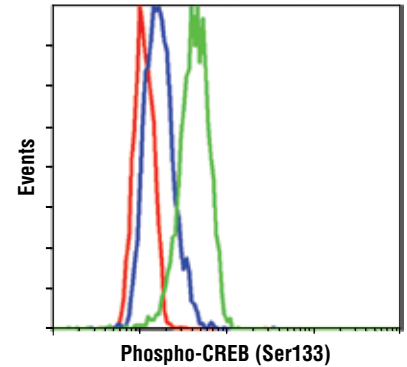
Immunohistochemical analysis of paraffin-embedded human renal cell carcinoma, untreated (left) or λ phosphatase-treated (right), using Phospho-CREB (Ser133) (87G3) Rabbit mAb.



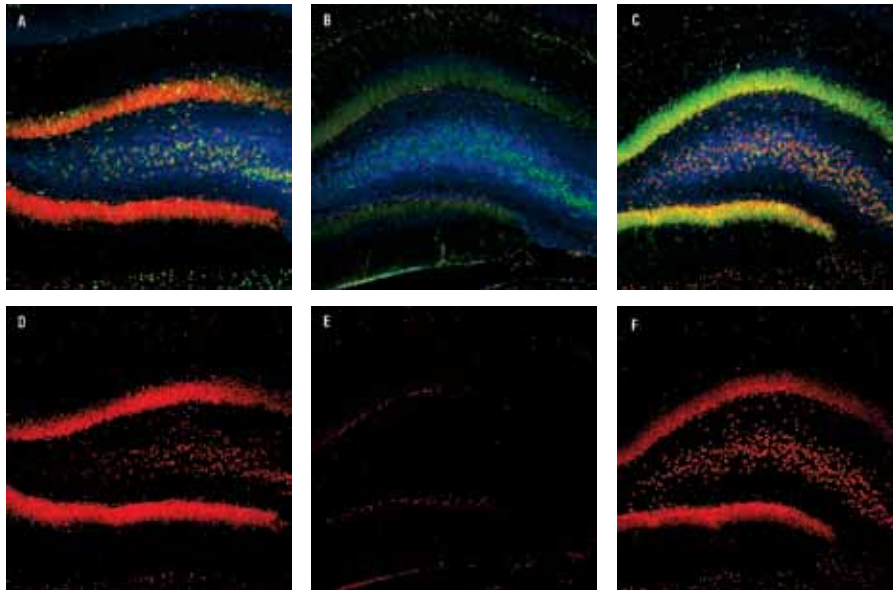
Immunohistochemical analysis of paraffin-embedded human breast carcinoma, using Phospho-CREB (Ser133) (87G3) Rabbit mAb in the presence of control peptide (left) or Phospho-CREB (Ser133) Blocking Peptide #1090 (right).

Background References:

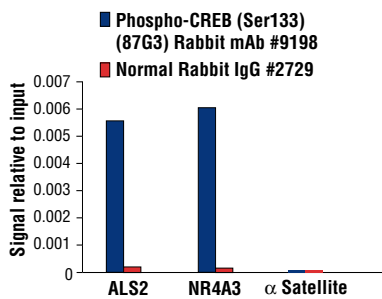
- (1) Lonze, B.E. et al. (2002) *Neuron* 34, 371–385.
- (2) Lee, M.M. et al. (1999) *J. Neurosci. Res.* 55, 702–712.
- (3) Redmond, L. et al. (2002) *Neuron* 34, 999–1010.
- (4) Dash, P.K. et al. (1990) *Nature* 345, 718–721.
- (5) Yin, J.C. et al. (1994) *Cell* 79, 49–58.
- (6) Guzowski, J.F. and McLaugh, J.L. (1997) *Proc. Nat. Acad. Sci. USA* 94, 2693–2698.
- (7) Xing, J. et al. (1998) *Mol. Cell. Biol.* 18, 1946–1955.
- (8) Ribar, T.J. et al. (2000) *J. Neurosci.* 20, RC107.
- (9) Tan, Y. et al. (1996) *EMBO J.* 15, 4629–4642.



Flow cytometric analysis of SK-N-MC cells, untreated (blue) or IBMX- and forskolin-treated (green), using Phospho-CREB (Ser133) (87G3) Rabbit mAb compared to a nonspecific negative control antibody (red).



Confocal immunofluorescent images of rat dentate gyrus, either sham-operated (left) or 15 min ischemia followed by 30 min (center) and 4 h (right) reperfusion, labeled with Phospho-CREB (Ser133) (87G3) Rabbit mAb (red), Neurofilament-L (DA2) Mouse mAb #2835 (blue) and Phospho-S6 Ribosomal Protein (Ser235/236) (2F9) Rabbit mAb (Alexa Fluor® 488 Conjugate) #4854.



◀ Chromatin immunoprecipitations were performed with cross-linked chromatin from 4×10^6 293 cells treated with Forskolin #3828 (30 μ M) for 1h and either 20 μ l of Phospho-CREB (Ser133) (87G3) Rabbit mAb or 2 μ l of Normal Rabbit IgG #2729 using SimpleChIP™ Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by Real-Time PCR using human ALS2 Exon 1 Primers, SimpleChIP™ Human NR4A3 Promoter Primers #4829, and SimpleChIP™ Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.