

#9190 Store at -20°C

PhosphoPlus® CREB (Ser133) Antibody Kit

✓ 10 western-blot



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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.

| Products Included | Product # | Quantity | Mol. Wt. | Isotype |
|---|-----------|----------|----------|------------|
| CREB (48H2) Rabbit mAb | 9197 | 100 µl | 43 kDa | Rabbit IgG |
| Phospho-CREB (Ser133) (87G3) Rabbit mAb | 9198 | 100 µl | 43 kDa | Rabbit IgG |
| CREB Control Cell Extracts | 9193 | 40 µl | | |
| Anti-rabbit IgG, HRP-linked Antibody | 7074 | 100 µl | | Goat |
| Anti-biotin, HRP-linked Antibody | 7075 | 100 µl | | Rabbit IgG |
| 20X LumiGLO® Reagent and 20X Peroxide | 7003 | 5 ml | | |
| Biotinylated Protein Ladder | 7727 | 100 µl | | |

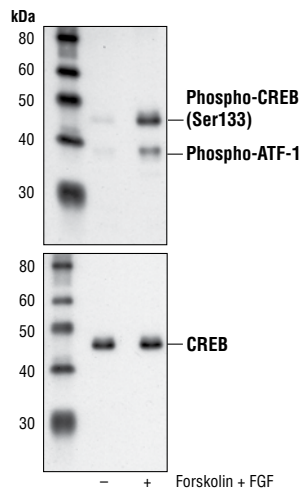
See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The PhosphoPlus CREB (Ser133) Antibody Kit provides reagents and protocols for the rapid analysis of the phosphorylation status of CREB at Ser133.

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, Ca²⁺ 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 Ser133. This antibody also detects the phosphorylated form of CREB-related protein ATF-1. CREB (48H2) Rabbit mAb detects endogenous levels of total CREB protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser133 of human CREB (Phospho-CREB (Ser133) (87G3) Rabbit mAb), or with a synthetic peptide corresponding to residues near the carboxy-terminus of human CREB (CREB (48H2) Rabbit mAb).



Western blot analysis of extracts from SK-N-MC cells, untreated or forskolin- and FGF-treated, using **Phospho-CREB (Ser133) (87G3) Rabbit mAb #9198** (upper) or **CREB (48H2) Rabbit mAb #9197** (lower).

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 antibodies.

Recommended Antibody Dilutions:
 Western blotting 1:1000

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 McGaugh, 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.

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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.

Selected rabbit monoclonal antibodies are produced under license (granting certain rights including those under U. S. Patent No. 5,675,063 and/or U.S.S.N. 11/476,277) from Epitomics, Inc. U.S.S.N. 11/476,277) from Epitomics, Inc.

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

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.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.