

#9190 Store at -20°C

# PhosphoPlus® CREB (Ser133) Antibody Kit

✓ 10 Western mini-blot



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

rev. 12/04/07

This product is for *in vitro* research use only and is not intended for use in humans or animals.

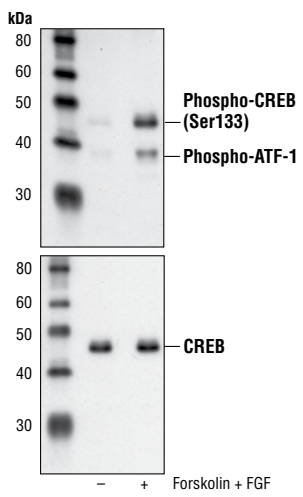
Products Included	Product #	Quantity	Applications	Species Cross-Reactivity	Mol. Wt.	Source
CREB (48H2) Rabbit mAb	9197	100 µl	W, IP, IHC-P, IHC-F, IF-F, IF-IC, F	H, M, R, Mk, Dr	43 kDa	Rabbit
Phospho-CREB (Ser133) (87G3) Rabbit mAb	9198	100 µl	W, IHC-P, IHC-F, IF-F, IF-IC, F	H, M, R	43 kDa	Rabbit
CREB Control Cell Extracts	9193	40 µl				
Anti-rabbit IgG, HRP-linked Antibody	7074	50 µl				Goat
Anti-biotin, HRP-linked Antibody	7075	100 µl				Goat
20X LumiGLO® Reagent and 20X Peroxide	7003	5 ml				
Biotinylated Protein Ladder	7727	100 µl				

**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, Ca<sup>2+</sup> 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:** Rabbit monoclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser133 of human CREB (Phospho-CREB (Ser133) (87G3) Rabbit mAb), or with a synthetic peptide (KLH-coupled) 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).

**Selected Application References:**

Constantinescu, A. et al. (2002) cAMP-dependent protein kinase types I and II differentially regulate cAMP response element-mediated gene expression: implications for neuronal responses to ethanol. *J. Biol. Chem.* 277, 18810-18816. Application: IC-ABC.

Liu, X. and Green, C.B. (2002) Circadian regulation of nocturnin transcription by phosphorylated CREB in *Xenopus* retinal photoreceptor cells. *Mol. Cell. Biol.* 22, 7501-7511. Application: IHC-FL (floating/frozen).

**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

See www.cellsignal.com for individual component dilutions and additional application protocols.

**Companion Products:**

- CREB (86B10) Mouse mAb #9104
- Phospho-CREB (Ser133) Antibody #9191
- CREB Control Cell Extracts #9193
- Phospho-CREB (Ser133) (1B6) Mouse mAb #9196
- CREB (48H2) Rabbit mAb #9197
- Phospho-CREB (Ser133) (87G3) Rabbit mAb #9198
- Phospho-CREB (Ser133) (87G3) Rabbit mAb (Alexa Fluor® 488 Conjugate) #9187
- Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder #7727

**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.

© 2007 Cell Signaling Technology, Inc. Rabbit Monoclonals produced using technology from Epitomics, Inc. under U.S. Patent No. 5,675,063.

**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected  
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

## 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.

- 1X Phosphate Buffered Saline (PBS)
- 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 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).
- Nonfat Dry Milk (weight to volume [w/v])
- 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%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- 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%).
- Phototope<sup>®</sup>-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<sup>®</sup> chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 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.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- 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.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- 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.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- 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.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

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

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

- 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<sup>®</sup> incubation and declines over the following 2 hours.