

# Phospho-Elk-1 (Ser383) (2B1) Mouse mAb

✓ 100 µl  
(20 Western mini-blot)

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

Applications	Species Cross-Reactivity*	Molecular Wt.	Source	Isotype
W	(H, M, R)	62 kDa	Mouse	IgG1

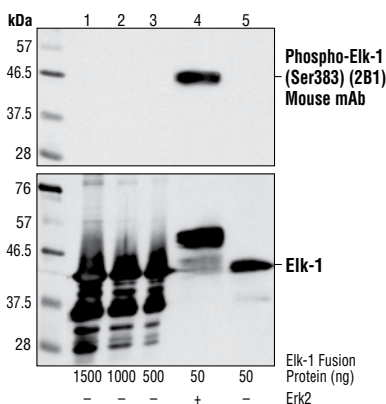
**Background:** The transcription factor Elk-1 is a component of the ternary complex that binds the serum response element (SRE) and mediates gene activity in response to serum and growth factors (1–3). Elk-1 is phosphorylated by MAP kinase pathways at a cluster of S/T motifs at its carboxy-terminus; phosphorylation at these sites, particularly Ser383, is critical for transcriptional activation by Elk-1. Elk-1 appears to be a direct target of activated MAP kinase: (a) biochemical studies indicate that Elk-1 is a good substrate for MAP kinase; (b) the kinetics of Elk-1 phosphorylation and activation correlate with MAP kinase activity; (c) interfering mutants of MAP kinase block Elk-1 activation *in vivo*. Other studies have shown that Elk-1 (Ser383) is also a target of the stress-activated kinase SAPK/JNK (4,5).

**Specificity/Sensitivity:** Phospho-Elk-1 (Ser383) (2B1) Mouse mAb detects less than 5 ng of phosphorylated Elk-1, and will not react with up to 1 µg of nonphosphorylated Elk-1 protein.

**Source/Purification:** Monoclonal antibody is produced by immunizing mice with a synthetic phospho-peptide (KLH-coupled) corresponding to residues around Ser383 of human Elk-1.

**Background References:**

- (1) Marais, R. et al. (1993) *Cell* 73, 381–393.
- (2) Kortjenann, M. et al. (1994) *Mol. Cell. Biol.* 14, 4815–4824.
- (3) Hill, C.S. and Treisman, R. (1995) *Cell* 80, 199–211.
- (4) Cavigelli, M. et al. (1995) *EMBO J.* 14, 5957–5964.
- (5) Whitmarsh, A.J. et al. (1995) *Science* 269, 403–407.



Western blot analysis of Elk-1 fusion protein expressed from *E. coli* with or without phosphorylation with purified Erk2 enzyme, using Phospho-Elk-1 (Ser383) (2B1) Mouse mAb (upper) or Elk-1 Antibody #9182 (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 antibody.

**Recommended Antibody Dilutions:**  
Western blotting 1:2000

- Companion Products:**  
PhosphoPlus® p44/42 MAP Kinase (Thr202/Tyr204) Antibody Kit #9100  
Phospho-p44/42 MAP Kinase (Thr202/Tyr204) Antibody #9101  
p44/42 MAP Kinase Antibody #9102  
Phospho-p44/42 MAPK (Thr202/Tyr204) (E10) Mouse mAb #9106  
p42 MAP Kinase (3A7) Mouse mAb #9107  
Phospho-Elk-1 (Ser383) Antibody #9181  
Elk-1 Antibody #9182  
Anti-mouse IgG, HRP-linked Antibody #7076  
Prestained Protein Marker, Broad Range (Premixed Format) #7720  
Biotinylated Protein Ladder Detection Pack #7727  
20X LumiGLO® Reagent and 20X Peroxide #7003

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

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence F—Flow cytometry E—ELISA D—DELFIATM  
**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 Milk)

For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 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% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7072:** Includes biotinylated protein ladder, secondary anti-mouse (#7076) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® 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 marker (#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® (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.

- 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.