

Elk-1 Antibody

100 µl
 (10 Western mini-blots)

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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
W Endogenous	H, M, R, Z	47 kDa	Rabbit

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).

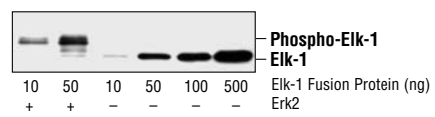
Specificity/Sensitivity: Elk-1 Antibody detects endogenous levels of total Elk-1 protein regardless of its phosphorylation state.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) derived from the sequence of human Elk-1. Antibodies are purified by protein A and peptide affinity chromatography.

Selected Application References:
 Aplin, A.E. et al. (2001) Integrin-mediated adhesion regulates ERK nuclear translocation and phosphorylation of Elk-1. *J. Cell Biol.* 153, 273–282.

Stewart, S. and Guan, K.L. (2000) The dominant negative Ras mutant, N17Ras, can inhibit signaling independently of blocking Ras activation. *J. Biol. Chem.* 275, 8854–8862.

Wang, D. and Richmond, A. (2001) Nuclear factor-κB activation by the CXC chemokine melanoma growth-stimulatory activity/growth-regulated protein involves the MEK1/p38 mitogen-activated protein kinase pathway. *J. Biol. Chem.* 276, 3650–3659.



Western blot analysis of Elk-1 fusion protein phosphorylated *in vitro* with purified Erk2 enzyme, using control Elk-1 Antibody.

Majka, M. et al. (2000) Stromal-derived factor 1 and thrombopoietin regulate distinct aspects of human megakaryopoiesis. *Blood* 96, 4142–4151.

Berman, D.E. et al. (1998) Specific and differential activation of mitogen-activated protein kinase cascades by unfamiliar taste in the insular cortex of the behaving rat. *J. Neurosci.* 18, 10037–10044.

Background References:

- (1) Marais, R. et al. (1993) *Cell* 73, 381–393.
- (2) Kortenjann, 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.

Entrez-Gene ID #2002
Swiss-Prot Acc. #P19419

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.

Recommended Antibody Dilutions:
 Western Blotting 1:1000

- Companion Products:**
- p44/42 MAP Kinase Assay Kit (nonradioactive) #9800
 - Phospho-Erk1/2 Pathway Sampler Kit #9911
 - PhosphoPlus® Elk-1 (Ser383) Antibody Kit #9180
 - Phospho-Elk-1 (Ser383) Antibody #9181
 - Elk-1 Control Proteins #9183
 - Phospho-Elk-1 (Ser383) (2B1) Mouse mAb #9186
 - PD98059 (MEK1 Inhibitor) #9900
 - U0126 (MEK1/2 Inhibitor) #9903
 - Anti-rabbit IgG, HRP-linked Antibody #7074
 - 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 BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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®-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.
- 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 cm²) 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.