#9180 Store at -20°C

PhosphoPlus® Elk-1 (Ser383) Antibody Kit

✓ 1 Kit
(10 western blots)

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-Elk-1 (Ser383) Antibody	9181	100 µl	47 kDa	Rabbit IgG
Elk-1 Antibody	9182	100 µl	47 kDa	Rabbit IgG
Elk-1 Control Proteins	9183	2 X 150 µl		
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-biotin, HRP-linked Antibody	7075	100 µl		Goat
Biotinylated Protein Ladder Detection Pack	7727	100 µl		
20X LumiGLO® Reagent and 20X Peroxide	7003	2 X 5 ml		

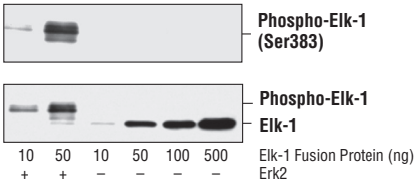
See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions, and additional application protocols.

Description: The PhosphoPlus® Elk-1 (Ser383) Antibody Kit provides reagents and protocols for the rapid analysis of the phosphorylation status of Elk-1. Bacterially expressed Elk-1 serves as a negative control. The same protein phosphorylated by p42 MAP Kinase (Erk2) serves as a positive control.

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. Phospho-Elk-1 (Ser383) Antibody detects Elk-1 protein only when activated by phosphorylation at serine 383.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phosphopeptide corresponding to residues surrounding Ser383 of human Elk-1, or with a synthetic peptide derived from the sequence of human Elk-1. Antibodies are purified by protein A and peptide affinity chromatography.



Western analysis of Elk-1 fusion protein expressed from E. coli with or without phosphorylation with purified Erk2 enzyme using ***Phospho-Elk-1 (Ser383) #9181*** *(upper) or* ***Elk-1 Antibodies #9182*** *(lower).*

Storage: Antibodies are 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.

Elk-1 Control Proteins are supplied in 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 phenol red. Store at –20°C.

Recommended Antibody Dilutions:

Western blotting	1:1000

Please visit www.cellsignal.com for a complete listing of recommended companion products.

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.

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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.

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 cm^2) 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.

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