

Protein Kinase Substrate

Elk-1 Fusion Protein

Concentration: 0.5 mg/ml

Recombinant

- Small 0.1 mg
- Large 0.5 mg

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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.

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).

Description: Elk-1 Fusion Protein serves as a useful substrate for p42/44 MAP kinases (1,6). It is expressed as a recombinant protein fusion containing Elk-1 residues 307-428.

Source/Purification: Cloned from a human cDNA library (7) and overexpressed in *E. coli*.

Quality Control: The purified fusion protein was identified by SDS-PAGE and Western blot analysis, using Elk-1 Antibody #9182, to be greater than 95% pure Elk-1.

Directions for Use: Elk 1 Fusion Protein, at a concentration of 2 µg/20 µl reaction, can be phosphorylated using 50 units of p42 MAP kinase (Erk2) in an *in vitro* kinase assay with 1X Kinase Buffer #9802 and 200 µM ATP #9804. After a 30-minute assay at 30°C, phosphorylation can be detected by Western blot with Phospho-Elk-1 (Ser383) Antibody #9181.

Molecular Weight: 41, 45 kDa

Specific Activity: Elk 1 Fusion Protein at a concentration of 2 µg/20 µl reaction was phosphorylated using p42 MAP kinase (Erk) in an *in vitro* kinase assay with 1X Kinase Buffer #9802 and 200 µM ATP #9804. After a 30-minute assay at 30°, phosphorylation was detected by Western blot with Phospho-Elk-1 (Ser383) Antibody #9181.

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) Cavignelli, M. et al. (1995) *EMBO J.* 14, 5957-5964.
- (5) Whitmarsh, A.J. et al. (1995) *Science* 269, 403-407.

Storage: Supplied in 20 mM Tris-HCl (pH 7.5 at 25°C), 50 mM NaCl, 2 mM Na2EDTA, 1 mM dithiothreitol (DTT) and 50% glycerol. Store at -20°C.

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

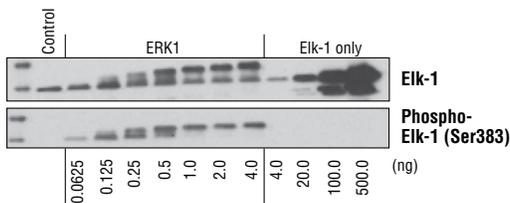


Figure 1. Western blot analysis of Elk-1 fusion protein phosphorylated by Erk1 kinase, using Elk-1 Antibody #9182 (upper panel) and Phospho-Elk-1 (Ser383) Antibody #9181 (lower panel).

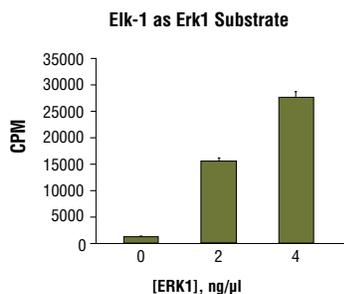


Figure 2. Elk-1 fusion protein was used as substrate to measure Erk1 kinases activity in a radiometric assay using the following reaction conditions: 25 mM Tris-HCl (pH7.5), 10 mM MgCl₂, 5 mM b-glycerophosphate, 0.1 mM Na₂VO₄, 2 mM DTT, 50 µM ATP; Substrate: Elk-1 fusion protein 400 ng/µL, and variable amounts of Erk1 kinases.

Fusion Protein Protocol

A Solutions and Reagents

- Note:** Prepare solutions with Milli-Q or equivalently purified water.
- 10X Kinase Buffer (#9802):** Store at -20°C . May be stored at 4°C for short-term use (1–2 weeks).
- Elk-1 Fusion Protein:** Concentration = 0.5mg/ml. Use 0.5 μg assay.
- 10 mM ATP Adenosine-5' triphosphate (ATP)** supplied as a 10 mM solution in sterile, doubly distilled water as a disodium salt. #9804
- ^{32}P ATP
- * **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH=8.5).
- * **3X SDS Sample Buffer:** 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v sodium dodecyl sulfate (SDS), 30% glycerol, 150 mM dithiothreitol (DTT), 0.03% w/v bromophenol blue. For 100 mL, use 2.27 g Tris-HCl, 6g SDS, 30 mL glycerol and 30mg w/v bromophenol blue or bromophenol blue dye. Store at -20°C . Add DTT fresh just before use.
- * **10X Tris-Buffered Saline with Tween-20 (TBS/T):** 0.2 M Tris base, 1.36 M NaCl, 1.0% Tween-20. To prepare 1 liter, dissolve 24.2 g Tris, 80 g NaCl in dH_2O and adjust pH to 7.6 with HCl. Store at room temperature.
- * **Blocking Buffer:** 1X TBS/T with 5% w/v nonfat dry milk. For 150 mL, dissolve 7.5g nonfat dry milk in 15 mL 10X TBS/T and 135 mL dH_2O . Mix well. Prepare freshly for each experiment.
- * **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T). Store at room temperature.
- * **Primary Antibody Dilution Buffer:** 1X TBS/T with 5% BSA.
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein marker, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP (#7075), 20X LumiGLO[®] chemiluminescent reagent and 20X peroxide (#7003).
- LumiGLO[®] Substrate #7003:** 0.5 mL 20X LumiGLO, 0.5 mL 20X peroxide and 9.0 mL Milli-Q water.

B Radiometric Assay Protocol

- Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 μM ATP.
- Dilute [^{32}P] ATP to 0.2 $\mu\text{Ci}/\mu\text{l}$ [^{32}P] ATP with 250 μM ATP solution.
- Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
- Dilute enzyme protein to desired concentration with 1X assay buffer followed by 2-fold serial dilutions.
- To start the reaction combine 10 μl kinase solution, 10 μl Elk-1 Fusion Protein (0.5 $\mu\text{g}/\mu\text{l}$) and 5 μl 0.2 $\mu\text{Ci}/\mu\text{l}$ [^{32}P] ATP solution.

Final Assay Conditions

25 mM Tris-HCl (pH 7.5)
 10 mM MgCl_2
 5 mM β -glycerophosphate
 0.1 mM Na_3VO_4
 2 mM DTT
 200 μM ATP
 200 $\mu\text{g}/\text{ml}$ Elk-1 fusion protein
 10–100 ng Kinase

- After 15 minutes terminate reaction by spotting 20 μl of the reaction mixture onto phosphocellulose P81 paper.
- Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
- Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
- Count samples in a scintillation counter.

C Kinase Assay

- Dilute 10 mM ATP with 2X kinase assay buffer by 1:25 to make 400 μM ATP.
- Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
- Dilute enzyme protein to desired concentration with 2X assay buffer (with ATP).
- To start the reaction combine 20 μl diluted kinase solution and Elk-1 (0.5 $\mu\text{g}/\mu\text{l}$).

Final Assay Conditions for a 40 μl Reaction

25 mM Tris-HCl (pH 7.5)
 10 mM MgCl_2
 5 mM β -glycerophosphate
 0.1 mM Na_3VO_4
 2 mM DTT
 200 μM ATP
 250 $\mu\text{g}/\text{ml}$ Elk-1 fusion protein
 10–100 ng Kinase

- After 30 minutes terminate reaction with 20 μl 3X SDS Sample Buffer.

D Western Immunoblotting

- Heat the sample to 95 – 100°C for 2–5 minutes.
- Load 5–15 μl of sample per well sample on SDS-PAGE gel.
- Note:** CST recommends loading prestained molecular weight markers (#7720, 10 $\mu\text{L}/\text{lane}$) to verify electrotransfer and biotinylated protein marker (#7727, 10 $\mu\text{L}/\text{lane}$) to estimate molecular weights.
- Run SDS-page and electrotransfer to nitrocellulose or PVDF membrane.
- Note:** Volumes for all the following steps are for 10 cm x 10 cm membrane; for different sized membranes, adjust volumes accordingly.
- (Optional) After transfer, wash nitrocellulose membrane with 25 mL TBST for 5 minutes at room temperature.
- Incubate membrane in 10 mL Blocking Buffer for 1–2 hours at room temperature.
- Wash three times for 5 minutes each with 15 mL Wash Buffer.
- Incubate membrane and Phospho-Elk-1 (Ser383) Antibody #9181 (1:1000 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 Wash Buffer.
- 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 Wash Buffer.
- Incubate membrane with 10 mL LumiGLO[®] Substrate with gentle agitation for 1 minute at room temperature.
- Drain membrane of excess LumiGLO[®] Substrate (but 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. LumiGLO[®] Substrate can be further diluted if signal response is too fast.