

SAPK/JNK Control Cell Extracts

✓ Controls for 10 Western mini-blot

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com

Support ■ 877-678-TECH (8324)
info@cellsignal.com

Web ■ www.cellsignal.com

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Background: The stress-activated protein kinase/Jun-amino-terminal kinase SAPK/JNK is potently and preferentially activated by a variety of environmental stresses, including UV and γ radiation, ceramides, inflammatory cytokines and in some instances, by growth factors and GPCR agonists (1-6). As with the other MAPKs, the core signaling unit is composed of a MAPKKK, typically MEKK1-4, or by one of the mixed lineage kinases (MLKs), which phosphorylate and activate MKK4-7, which then phosphorylate and activate the SAPK/JNK kinase (2). Stress signals are delivered to this cascade by small GTPases of the Rho family (Rac, Rho, cdc42) (3). Both Rac1 and cdc42 mediate the stimulation of MEKs and MLKs (3). Alternatively, MKK4-7 can be activated by a pathway independent of small GTPases via stimulation of a member of the germinal center kinase (GCK) family (4). There are three SAPK/JNK genes with further diversification resulting from alternative splicing (3). SAPK/JNK, when active as a dimer, can translocate to the nucleus where it regulates transcription through its effects on c-Jun, ATF-2 and other transcription factors (3,5).

Description:

Nonphosphorylated SAPK/JNK Control Cell Extracts: Total cell extracts from 293 cells, prepared without treatment, serve as a negative control. Supplied in SDS Sample Buffer.

Phosphorylated SAPK/JNK Control Cell Extracts: Total cell extracts from 293 cells, treated with UV light, serve as a positive control. Supplied in SDS Sample Buffer.

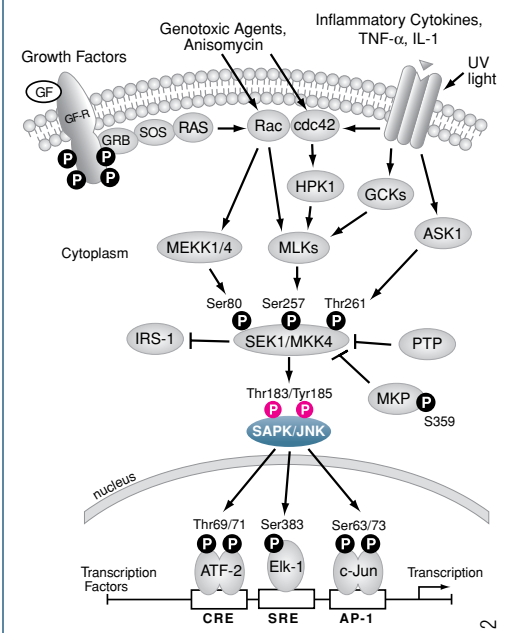
Applications: As controls, we recommend using 15 μ l of phosphorylated and nonphosphorylated SAPK/JNK control extracts. Boil 2 minutes prior to use.

Background References:

- (1) Davis, R.J. (1999) *Biochem. Soc. Symp.* 64, 1-12.
- (2) Ichijo, H. (1999) *Oncogene* 18, 6087-6093.
- (3) Kyriakis, J.M. and Avruch, J. (2001) *Physiol. Rev.* 81, 807-869.
- (4) Kyriakis, J.M. (1999) *J. Biol. Chem.* 274, 5259-5262.
- (5) Leppa, S. and Bohmann, D. (1999) *Oncogene* 18, 6158-6162.
- (6) Whitmarsh, A.J. and Davis, R.J. (1998) *Trends Biochem. Sci.* 23, 481-485.

Companion Products:

PhosphoPlus® SAPK/JNK (Thr183/Tyr185) Antibody Kit #9250
Phospho-SAPK/JNK (Thr183/Tyr185) Antibody #9251
SAPK/JNK Antibody #9252
Phospho-SAPK/JNK (Thr183/Tyr185) (G9) Mouse mAb #9255
Phospho-SAPK/JNK (Thr183/Tyr185) (81E11) Rabbit mAb #4668
SAPK/JNK (56G8) Rabbit mAb #9258
Phospho-SAPK/JNK(T183/Y185)(98F2) Rabbit mAb #4671
Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
Anti-rabbit IgG, HRP-linked Antibody #7074
Prestained Protein Marker, Broad Range (Premixed Format) #7720
Biotinylated Protein Ladder #7727
20X LumiGLO® Reagent and 20X Peroxide #7003



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.