#6093 Store at -20°C

Protein Kinase Substrate

c-Jun Fusion Protein

Concentration: 1 mg/ml

Recombinant

☐ Small 0.1 mg

☐ Large 0.5 mg

rev. 02/09/16

Cell Signaling TECHNOLOGY®

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

Entrez-Gene ID #3725
Swiss-Prot Acc. #P05412

Background: c-Jun is a member of the Jun Family containing c-Jun, JunB and JunD, and is a component of the transcription factor AP-1 (activator protein-1). AP-1 is composed of dimers of Fos, Jun and ATF family members and binds to and activates transcription at TRE/AP-1 elements (reviewed in 1). Extracellular signals including growth factors, chemokines and stress activate AP-1-dependent transcription. The transcriptional activity of c-Jun is regulated by phosphorylation at Ser63 and Ser73 through SAPK/JNK (reviewed in 2). Knock-out studies in mice have shown that c-Jun is essential for embryogenesis (3), and subsequent studies have demonstrated roles for c-Jun in various tissues and developmental processes including axon regeneration (4), liver regeneration (5) and T cell development (6). AP-1 regulated genes exert diverse biological functions including cell proliferation, differentiation, and apoptosis, as well as transformation, invasion and metastasis, depending on cell type and context (7-9). Other target genes regulate survival as well as hypoxia and angiogenesis (8,10). c-Jun has emerged as a promising therapeutic target for cancer, vascular remodeling, acute inflammation, as well as rheumatoid arthritis (11,12).

Description: c-Jun Fusion Protein serves as a useful substrate for SAPK/JNK, which will phosphorylate it at Ser63 and Ser73 (1). It is expressed as a recombinant protein fusion to amino acid residues corresponding to c-Jun codons 1-89.

Source/Purification: Isolated from a strain of E. coli that carries the coding sequence for human c-Jun residues 1-89 (kindly provided by Dr. J.R. Woodgett). Protein was purified by affinity chromatography.

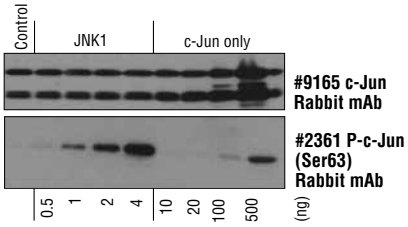

Figure 1. Western blot analysis of c-Jun fusion protein phosphorylated by JNK1 kinase, using c-Jun (60A8) Rabbit mAb #9165 (upper panel) and Phospho-c-Jun (Ser63) (54B3) Rabbit mAb #2361 (lower panel).

Quality Control: The purified protein was identified by SDS-PAGE and Western blot analysis, using c-Jun Antibody #9162, to be greater than 95% c-Jun Fusion Protein.

Directions for Use: c-Jun Fusion Protein at a concentration of 0.5 µg/µl in a 20 µl reaction can be phosphorylated using active SAPK 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-specific c-Jun antibodies (#9164 and #9261).

Molecular Formula: Apparent Molecular Weight: 37 kDa

Selected Application References:

Malek, R.L. et al. (2001) Nrg-1 belongs to the endothelial differentiation gene family of G protein-coupled sphingosine-1-phosphate receptors. *J. Biol. Chem.* 276, 5692–5699.

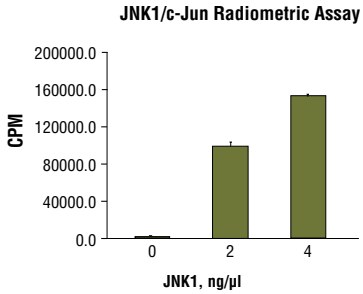

Figure 2. c-Jun fusion protein was used as substrate to measure JNK1 kinases activity in a radiometric assay using the following reaction conditions: 25 mM Tris-HCl (pH7.5), 10 mM MgCl2, 5 mM b-glycerophosphate, 0.1 mM Na3VO4, 2 mM DTT, 50 µM ATP, Substrate: c-Jun fusion protein 400 ng/µL, and variable amounts of JNK1 kinases.

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.

For application specific protocols please see the web page for this product at www.cellsignal.com.

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

Background References:

(1) Jochum, W. et al. (2001) *Oncogene* 20, 2401–12.
(2) Davis, R.J. (2000) *Cell* 103, 239–52.
(3) Hilberg, F. et al. (1993) *Nature* 365, 179–81.
(4) Raivich, G. et al. (2004) *Neuron* 43, 57–67.
(5) Behrens, A. et al. (2002) *EMBO J* 21, 1782–90.
(6) Riera-Sans, L. and Behrens, A. (2007) *J Immunol* 178, 5690–700.
(7) Leppä, S. and Bohmann, D. (1999) *Oncogene* 18, 6158–62.
(8) Shaulian, E. and Karin, M. (2002) *Nat Cell Biol* 4, E131–6.
(9) Weiss, C. and Bohmann, D. (2004) *Cell Cycle* 3, 111–3.
(10) Karamouzis, M.V. et al. (2007) *Mol Cancer Res* 5, 109–20.
(11) Kim, S. and Iwao, H. (2003) *J Pharmacol Sci* 91, 177–81.
(12) Weiss, C. and Bohmann, D. (2004) *Cell Cycle* 3, 111–3.
(13) Dass, C.R. and Choong, P.F. (2008) *Pharmazie* 63, 411–4.

Fusion Protein Protocol

A Solutions and Reagents

1. **Note:** Prepare solutions with Milli-Q or equivalently purified water.
2. **10X Kinase Buffer (#9802):** Store at –20°C. May be stored at 4°C for short-termuse (1–2 weeks).
3. **c-Jun Fusion Protein:** Concentration = 2mg/ml.
4. **10 mM ATP Adenosine-5' triphosphate (ATP)** supplied as a 10 mM solution in sterile, doubly distilled water as a disodium salt. #9804
5. ^{32}ATP
6. * **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH=8.5).
7. * **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.
8. * **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.
9. * **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.
10. * **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T). Store at room temperature.
11. * **Primary Antibody Dilution Buffer:** 1X TBS/T with 5% BSA.
12. **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).
13. **LumiGLO® Substrate #7003:** 0.5 mL 20X LumiGLO, 0.5 mL 20X peroxide and 9.0 mL Milli-Q water.

B Radiometric Assay Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 µM ATP.
2. Dilute c-Jun (1 mg/ml) with dH_2O by 1:2 to make 0.5 mg/ml c-Jun solution.
3. Dilute [^{32}p] ATP to 0.2 µCi/µl [^{32}p] ATP with 250 µM ATP solution.
4. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
5. Dilute enzyme protein to desired concentration with 1X assay buffer followed by 2-fold serial dilutions.
6. To start the reaction combine 10 µl kinase solution, 10 µl c-Jun Fusion Protein (0.5 µg/µl) and 5 µl 0.2 µCi/µl [^{32}p] ATP solution.

Final Assay Conditions

25 mM Tris-HCl (pH 7.5)
10 mM $MgCl_2$
5 mM b-glycerophosphate
0.1 mM Na_3VO_4
2 mM DTT
200 µM ATP
200 µg/ml c-Jun fusion protein
10-100 ng Kinase

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

C Kinase Assay

1. Dilute 10 mM ATP with 2X kinase assay buffer by 1:25 to make 400 µM ATP.
2. Dilute c-Jun (1 mg/ml) with dH_2O by 1:2 to make 0.5 mg/ml c-Jun solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute enzyme protein to desired concentration with 2X assay buffer (with ATP).
5. To start the reaction combine 20 µl diluted kinase solution and 20 µl c-Jun (0.5 µg/µl).

Final Assay Conditions for a 40 µl Reaction

25 mM Tris-HCl (pH 7.5)
10 mM $MgCl_2$
5 mM b-glycerophosphate
0.1 mM Na_3VO_4
2 mM DTT
200 µM ATP
250 µg/ml c-Jun fusion protein
10-100 ng Kinase

6. After 30 minutes terminate reaction with 20 µl 3X SDS Sample Buffer.
7. Dilute sample with 1X SDS Sample Buffer by 1:167 to make 1 ng/µl c-Jun.

D Western Immunoblotting

1. Heat the sample to 95–100°C for 2–5 minutes.
2. Load 20 µl of sample per well sample on SDS-PAGE gel.
3. **Note:** CST recommends loading prestained molecular weight markers (#7720, 10 µL/lane) to verify electrotransfer and biotinylated protein marker (#7727, 10 µL/lane) to estimate molecular weights.
4. Run SDS-page and electrotransfer to nitrocellulose or PVDF membrane.
5. **Note:** Volumes for all the following steps are for 10 cm x 10 cm membrane; for different sized membranes, adjust volumes accordingly.
6. (Optional) After transfer, wash nitrocellulose membrane with 25 mL TBST for 5 minutes at room temperature.
7. Incubate membrane in 10 mL Blocking Buffer for 1-2 hours at room temperature.
8. Wash three times for 5 minutes each with 15 mL Wash Buffer.
9. Incubate membrane with Phospho-c-Jun (Ser63) Antibody #9261 (1:1000 dilution) in 10 mL Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
10. Wash three times for 5 minutes each with 15 mL Wash Buffer.
11. 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.
12. Wash three times for 5 minutes each with 15 mL Wash Buffer.
13. Incubate membrane with 10 mL LumiGLO® Substrate with gentle agitation for 1 minute at room temperature.
14. 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.
15. **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.

MSDS

Material Safety Data Sheet (MSDS) for Fusion Proteins

Cell Signaling TECHNOLOGY®

rev. 05/16/08

I. Identification:

Product name: Fusion Proteins
Product Catalog Number: 6000, 7000, and 9000 series
CAS number: None

Manufacturer Supplier: Cell Signaling Technology
3 Trask Lane
Danvers, MA 01923 USA
1-978-867-2300 TEL
1-978-867-2400 FAX
1-978-578-6737 Emergency Phone

II. Composition/Information on Ingredients:

This product is composed of proteins in aqueous buffer solution. According to 29 CFR 1910.1200(d), hazardous ingredients at less than <1% and carcinogens at less than < 0.1% are considered non-hazardous. This product may contain the following hazardous ingredients:

Ingredient	CAS#	Percent
Glycerol	56-81-5	50%

III. Hazard Identification:

To the best of our knowledge, the chemical, physical, and toxicological properties of this solution have not been thoroughly investigated.

Emergency Overview of Hazardous ingredient: Glycerol (CAS# 56-81-5)

Caution: Avoid contact and inhalation.
Target organ: Kidneys.

IV. First Aid Measures:

Inhalation: If inhaled, remove to fresh air. If breathing is difficult, get medical attention.
Ingestion: If swallowed, rinse mouth with water provided person is conscious. Get medical attention.
Skin Exposure: In case of contact, wash skin with soap and water.
Eye Exposure: In case of contact with eyes, immediately flush eyes with water for at least 15 minutes. Get medical attention.

V. Fire Fighting Measures:

Flash Point: Data not available.
Autoignition Temperature: Data not available.
Fire Extinguishing Media: Water spray, dry chemical, foam, or carbon dioxide.
Firefighting: Wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes.

VI. Accidental Release Measures:

Absorb liquid with an absorbent material. Transfer contaminated absorbent to a chemical waste container for disposal.

VII. Handling And Storage:

Avoid inhalation and contact with eyes and skin. Avoid prolonged or repeated exposure. Store at–20°C in tightly closed container.

VIII. Exposure Controls/Personal

Engineering Controls: Maintain adequate ventilation, eye wash and quick-drench facilities in work area.
Personal Protective Equipment: Lab coat, chemical resistant gloves and chemical safety glasses.
Occupational Exposure Limits: Data not available.

IX. Exposure Controls/Personal Protection:

Physical State:	liquid
Appearance:	colorless
Odor:	odorless
Boiling Point:	data not available
Melting Point:	data not available
Volatile Organic Compound:	data not available
Solubility in water:	readily miscible in water

X. Stability and Reactivity:

Stability: Stable under recommended conditions.
Hazardous Decomposition: May form carbon dioxide and carbon monoxide.
Conditions to avoid: Strong oxidizing agents.

XI. Toxicological Information:

May cause skin irritation.
May be toxic if absorbed through skin or ingested.
May cause eye irritation.

Target Organs: Kidneys
Prolonged exposure may cause nausea, headache, and vomiting.

XII. Ecological Information:

Data not available.

XIII. Disposal Considerations:

Dispose of in accordance with federal, state and local environmental regulations.

XIV. Transport Information:

D.O.T.: This substance is considered non-hazardous for transport.
IATA: This substance is considered non-hazardous for air transport.

XV. Regulatory Information:

US Classification and Label information:
Caution: Avoid contact and inhalation.
Target organ(s): Kidneys.

Chemical inventory status : Not classified/controlled according to EU, USA, WHMIS.

XVI. Other Information:

This product is not intended for use in humans. It is sold only for research use only. No other use is intended, and any other use may involve substantive hazards.

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide for experienced personnel. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product. The burden of safe use of this material rests entirely with the user.