Store at –20°C

#9260

PhosphoPlus® c-Jun (Ser63) and c-Jun (Ser73) Antibody Kit

☑ 1 Kit
(10 western blots)

Cell Signaling TECHNOLOGY®

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rev. 01/22/15

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

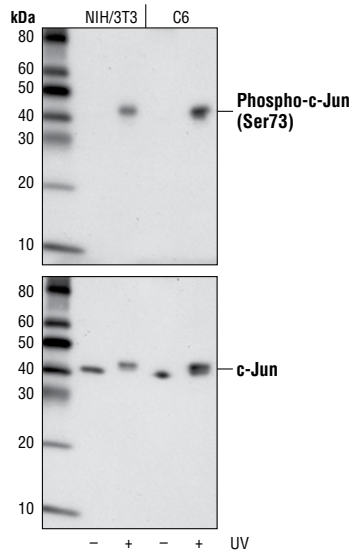
Products Included	Product #	Quantity	Mol. Weight	Isotype
Phospho-c-Jun (Ser63) (54B3) Rabbit mAb	2361	100 µl	48 kDa	Rabbit IgG
Phospho-c-Jun (Ser73) (D47G9) XP® Rabbit mAb	3270	100 µl	48 kDa	Rabbit IgG
c-Jun (60A8) Rabbit mAb	9165	100 µl	43, 48 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-biotin, HRP-linked Antibody	7075	100 µl		Goat
20X LumiGLO® Reagent and 20X Peroxide	7003	5 ml		
Biotinylated Protein Ladder	7727	100 µl		
c-Jun Control Cell Extracts	9263	150 µl		

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

Description: The PhosphoPlus® c-Jun (Ser63) and c-Jun (Ser73) Antibody Kit provided reagents and controls for rapid analysis of c-Jun phosphorylation status.

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).

Specificity/Sensitivity: Phospho-c-Jun (Ser63) (54B3) Rabbit mAb detects endogenous levels of c-Jun only when phosphorylated at Ser63. Phospho-c-Jun (Ser73) Antibody detects endogenous levels of c-Jun only when phosphorylated at Ser73. This antibody also recognizes phosphorylation of JunD at Ser100. c-Jun (60A8) Rabbit mAb detects endogenous levels of total c-Jun protein.



Western blot analysis of extracts from NIH/3T3 or C6 cells, untreated or UV-treated, using ***Phospho-c-Jun (Ser73) (D47G9) XP® Rabbit mAb #3270*** *(upper) or* ***c-Jun (60A8) Rabbit mAb #9165*** *(lower).*

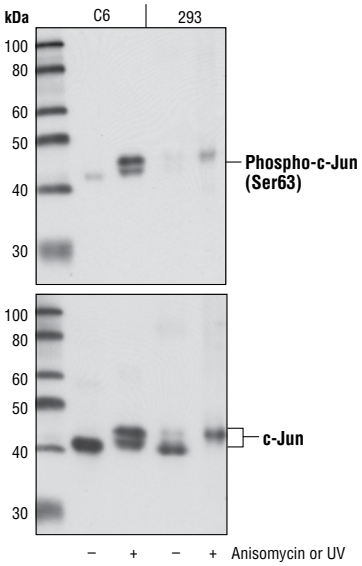
Source/Purification: Phospho-specific polyclonal antibodies are produced by immunizing rabbits with a synthetic phosphopeptide corresponding to residues surrounding Ser63 or Ser73 of human c-Jun, and purified by protein A and peptide affinity chromatography. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the amino-terminal of human c-Jun.

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 antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

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) Dass, C.R. and Choong, P.F. (2008) *Pharmazie* 63, 411-4.



Western blot analysis of extracts from untreated or anisomycin-treated C6 cells, or untreated or UV-treated 293 cells, using ***Phospho-c-Jun (Ser63) (54B3) Rabbit mAb #2361*** *(upper) or* ***c-Jun (60A8) Rabbit mAb #9165*** *(lower).*

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.

#9260

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.