

#9260 Store at -20°C

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

1 Kit
 (3 x 100 µl)



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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.

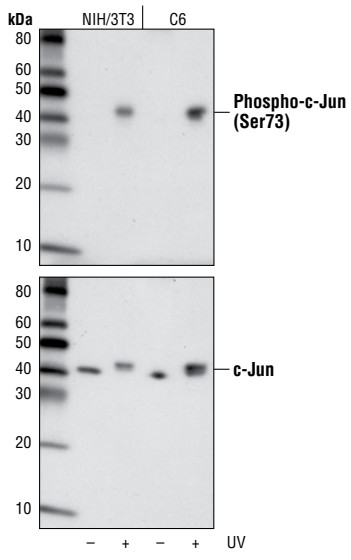
Products Included	Product #	Quantity	Mol. Weight	Isotype
Phospho-c-Jun (Ser63) (54B3) Rabbit mAb	2361	100 µl	48 kDa	Rabbit IgG
Phospho-c-Jun (Ser73) (D47G9) XP® Rabbit mAb	3270	100 µl	48 kDa	Rabbit IgG
c-Jun (60A8) Rabbit mAb	9165	100 µl	43, 48 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074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



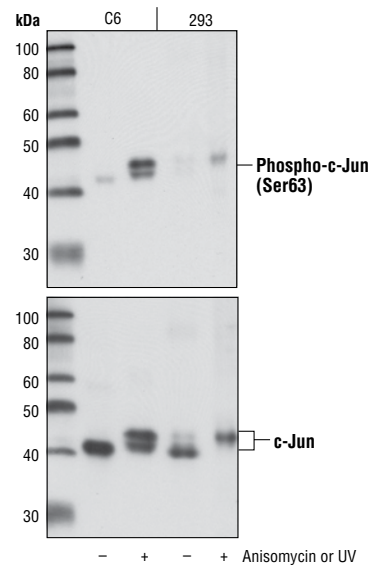
Western blot analysis of extracts from NIH/3T3 or C6 cells, untreated or UV-treated, using **Phospho-c-Jun (Ser73) (D47G9) XP® Rabbit mAb #3270** (upper) or **c-Jun (60A8) Rabbit mAb #9165** (lower).

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).

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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.

- 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.