Store at 4°C

#7141

PathScan® Phospho c-Jun (Ser63) Sandwich ELISA Antibody Pair

☑ 1 Kit
(4 X 96 assays)

Cell Signaling TECHNOLOGY®

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

Species Cross-Reactivity: H, M, R

Description: CST's PathScan® Phospho-c-Jun (Ser63) Sandwich ELISA Antibody Pair is being offered as an alternative to our PathScan® Phospho-c-Jun (Ser63) Sandwich ELISA Kit #7145. Capture and Detection antibodies (100X stocks) and a HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are provided for performing 4 x 96 well ELISAs. Phospho-c-Jun (Ser63) Capture Antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysates are added, followed by c-Jun Detection Antibody and HRP-conjugated secondary antibody. HRP substrate, TMB, is added for color development. The magnitude of the absorbance at 450 nm is proportional to the quantity of phospho-c-jun (Ser63) protein.

Reagents not supplied:
Phosphate Buffered Saline (PBS-20X) #9808

Phosphate Buffered Saline with Tween -20 (PBST-20X) #9809

Cell Lysis Buffer (10X) #9803

TMB Substrate #7004

STOP Solution #7002

Blocking Buffer- PBS+0.05% Tween®20, 1% BSA

96 Well Microplates**

Microplate Reader

** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592).

Note: Antibody pairs have been optimized using recommended buffers, reagents, plates and protocol. Solutions should be made fresh daily.

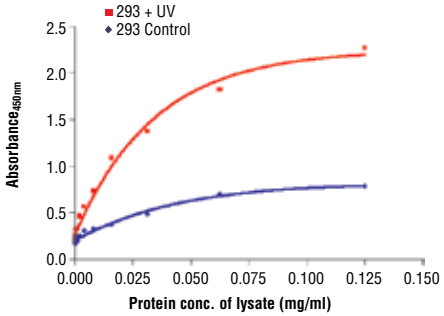
Background: c-Jun is a member of the Jun Family containing c-Jun, JunB and JunD, and is a component of the transcription factor AP-1 (activator protein-1). AP-1 is composed of dimers of Fos, Jun and ATF family members and binds to and activates transcription at TRE/AP-1 elements (Reviewed in 1).

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 promising therapeutic target for cancer, vascular remodeling, acute inflammation, as well as rheumatoid arthritis (11-13).

Products Included	Item #	Volume	Cap Color	Storage
Phospho-c-Jun (Ser63) Capture Rabbit mAb (100X)	59566	400 µL	Pink	4°C
c-Jun Detection Mouse mAb (100X)	63301	400 µL	Blue	4°C
Anti-mouse IgG, HRP-linked Antibody (1000X)	16736	40 µL	Yellow	–20°C

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



The relationship between lysate protein concentration from untreated and UV treated 293 cells and the absorbance at 450 nm using PathScan® Phospho-c-Jun (Ser 63) Sandwich ELISA Antibody Pair #7141 is shown. 293 cells were UV-treated, allowed to recover for 30 minutes at 37°C and then lysed.

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.

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.

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PathScan® Sandwich ELISA Antibody Pair Protocol

A Required Reagents

1. **Coating Buffer:** 1X PBS, (20X PBS #9808)
   3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, pH 7.4
2. **Wash Buffer:** 1X PBS/0.05% Tween®20, (20X PBST #9809)
3. **Blocking Buffer:** 1X PBS/0.05% Tween®20, 1% BSA
4. **1X Cell Lysis Buffer:** (10X Cell Lysis Buffer #9803)
   20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM ethylene diamine tetraacetate (EDTA), 1 mM ethylene glycol-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 µg/ml leupeptin.
5. **TMB Substrate:** (TMB Substrate #7004)
6. **STOP Solution:** (STOP Solution #7002)

NOTE: Reagents should be made fresh daily

B Coating Procedure

1. Rinse microplate with dH_2O. Add 200 µl of dH_2O and discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in PBS. For a single 96 well plate, add 100 µl of Capture Antibody Stock to 9.9 ml PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17-20 hours).
3. **After overnight coating, gently uncover plate and wash wells:**
   a. Discard plate contents into a receptacle.
   b. Wash 4 times with Wash Buffer, 200 µl each time for each well. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
   c. Clean the underside of all wells with a lint-free tissue.
4. Block plates. Add 150 µl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
5. After blocking, wash plate as in Step 3. Plate is ready to use.

C Preparing Cell Lysates

1. Aspirate media, treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm^2) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

D Test Procedure

1. Lysates can be used undiluted or diluted in Blocking Buffer. 100 µl of lysate is added per well. Cover plate and incubate at 37°C for 2 hours.
2. Wash plate as in Coating Procedure, Step 3.
3. Dilute detection antibody 1:100 in Blocking Buffer. For a single 96 well plate, add 100 µl of Detector Antibody Stock to 9.9 ml of Blocking Buffer. Mix well and add 100 µl/well. Cover plate and incubate at 37°C for 1 hour.
4. Plate is washed as in Coating Procedure, Step 3.
5. Secondary antibody, either, anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in Blocking Buffer. For a single 96 well plate, add 10 µl of secondary antibody stock to 9.99 ml of Blocking Buffer. Mix well and add 100 µl/well. Cover and incubate at 37°C for 30 minutes.
6. Wash plate as in Coating Procedure, Step 3.
7. Add 100 µl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
8. Add 100 µl of STOP Solution per well.
9. Read plate on a microplate reader at Absorbance 450 nm.