

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

✓ 1 Kit
(4 X 96 assays)



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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.

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

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

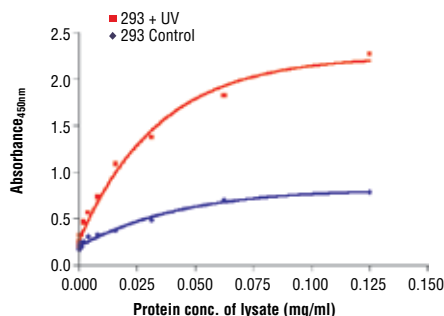
** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592) and Corning® 96 Well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene High Bind Microplates (#3369).

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

Products Included	Volume	Cap Color	Storage
Phospho-c-Jun (Ser63) Capture Antibody (100X)	0.4 ml	Pink	4°C
c-Jun Detection Antibody (100X)	0.4 ml	Blue	4°C
Anti-Mouse IgG HRP-linked (1000X)	0.04 ml	Natural	-20°C



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.

Storage: Phospho-c-Jun Capture Antibody and c-Jun Detection Antibody are stored at 4°C.

Anti-Mouse IgG-HRP Linked Antibody is stored at -20°C.

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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).

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.

PathScan® Sandwich ELISA Antibody Pair Protocol

A Required Reagents

- Coating Buffer:** 1X PBS, (20X PBS #9808)
3.2 mM Na₂HPO₄, 0.5 mM KH₂PO₄, 1.3 mM KCl, 135 mM NaCl, pH 7.4
- Wash Buffer:** 1X PBS/0.05% Tween-20, (20X PBST #9809)
- Blocking Buffer:** 1X PBS/0.05% Tween-20, 1% BSA
- 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 Na₃VO₄, 1 μg/ml leupeptin.
- TMB Substrate:** (TMB Substrate #7004)
- STOP Solution:** (STOP Solution #7002)

NOTE: Reagents should be made fresh daily

B Coating Procedure

- Rinse microplate with dH₂O. Add 200 μl of dH₂O and discard liquid. Blot on paper towel to make sure wells are dry.
- 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).
- After overnight coating, gently uncover plate and wash wells:**
 - Discard plate contents into a receptacle.
 - 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.
 - Clean the underside of all wells with a lint-free tissue.
- Block plates. Add 150 μl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
- After blocking, wash plate as in Step 3. Plate is ready to use.

C Preparing Cell Lysates

- Aspirate media, treat cells by adding fresh media containing regulator for desired time.
- To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenyl-methylsulfonyl fluoride (PMSF) to each plate (10 cm²) and incubate the plate on ice for 5 minutes.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- 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

- 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.
- Wash plate as in Coating Procedure, Step 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.
- Plate is washed as in Coating Procedure, Step 3.
- 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.
- Wash plate as in Coating Procedure, Step 3.
- Add 100 μl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
- Add 100 μl of STOP Solution per well.
- Read plate on a microplate reader at Absorbance 450 nm.