#7260 Store at 4°C

PathScan® Phospho-c-Jun (Ser63) Sandwich ELISA Kit

☑1 Kit (96 assays)

rev. 08/23/04

Cell Signaling TECHNOLOGY®

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Species Cross-Reactivity: H, M, R

Introduction: CST's PathScan® Phospho-c-Jun (Ser63) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of phospho-c-Jun (Ser63) protein. Phospho-c-Jun (Ser63) II Antibody (#9261*) has been coated onto the microwells. After incubation with cell lysates, phospho-c-Jun (Ser63) protein is captured by the coated antibody. Following extensive washing, c-Jun (6A3*) monoclonal antibody is added to detect the captured phospho-c-Jun protein. HRP-linked anti-mouse antibody (#7076*) is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of phospho-c-Jun (Ser63) protein.

*Antibodies are custom formulations specific to the kit

Companion Products:

PathScan® Total c-Jun Sandwich ELISA Kit #7270

Phospho-c-Jun (Ser63) II Antibody #9261

Anti-mouse IgG, HRP-linked Antibody #7076

Cell Lysis Buffer (10X) #9803

Specificity/Sensitivity: CST's PathScan® Phospho-c-Jun (Ser63) Sandwich ELISA Kit detects endogenous levels of Phospho-c-Jun (Ser63) protein. Using this Sandwich ELISA Kit #7260, a significant induction of phospho-c-Jun (Ser63) in 293 cells treated with UV light is detected. However, the level of total c-Jun, detected by the Total c-Jun Sandwich ELISA Kit #7270, remains unchanged (figure 1). Both C6 and NIH/3T3 cells treated with either UV light or anisomycin show similar results (data not shown).

Background: c-Jun is a component of the transcription factor AP-1, which binds and activates transcription at TRE/AP-1 elements. The transcriptional activity of c-Jun is regulated by phosphorylation at serine 63 and serine 73 (1,2). Extracellular signals, including growth factors, transforming oncoproteins and UV irradiation, stimulate phosphorylation of c-Jun at serine 63/73 and activate c-Jun-dependent transcription. Mutation of serine 63/73 renders c-Jun nonresponsive to mitogenic and stress-induced signaling pathways. The MAP kinase homologue SAPK/JNK binds to the amino-terminal region of c-Jun and phosphorylates c-Jun at serine 63/73. In addition, the activity of SAPK/JNK is stimulated by the same signals that activate c-Jun (3,4).

Kit Includes

Products	Volume	Solution Color
Phospho-c-Jun (Ser63) Ab coated Microwells*	96 tests	
c-Jun (6A3) Monoclonal Detection Ab	11 ml	green
Anti-mouse IgG HRP-Linked Ab	11 ml	red
TMB Substrate	11 ml	colorless
STOP Solution	11 ml	colorless
Sealing Tape	2 sheets	
20X Wash Buffer	25 ml	colorless
Sample Diluent	25 ml	blue
10X Cell Lysis Buffer #9803**	15 ml	yellowish

*12 8-well modules -Each module is designed to break apart for 8 tests.

**Kit should be stored at 4°C wtih the exception of 10X Cell Lysis Buffer, which is stored at –20°C (packaged separately).

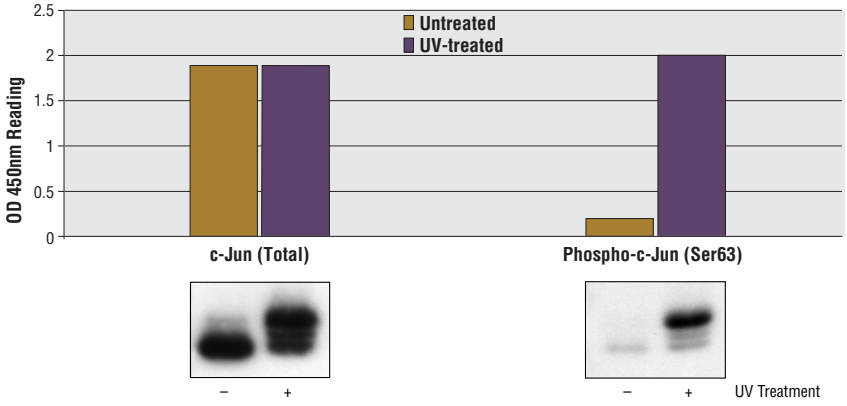

Figure 1: Treatment of 293 cells with UV light stimulates phosphorylation of c-Jun at Ser63, detected by PathScan® Phospho-c-Jun (Ser63) Sandwich ELISA kit, #7260, but does not affect the level of total c-Jun protein, detected by PathScan® Total c-Jun Sandwich ELISA kit, #7270. OD450 readings are shown in the top figure, while the corresponding Western blot using Phospho-c-Jun (Ser63) II Ab #9261 (right panel) or c-Jun Rabbit Monoclonal Antibody (6H5) (left panel), is shown in the bottom figure.

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Background References:

(1) Binetruy, B. et al. (1991) *Nature* 351, 122–127.
(2) Smeal, T. et al. (1991) *Nature* 354, 494–496.
(3) Derijard, B. et al. (1994) *Cell* 76, 1025–1037.
(4) Kyriakis, J. M. et al. (1994) *Nature* 369, 156–160.

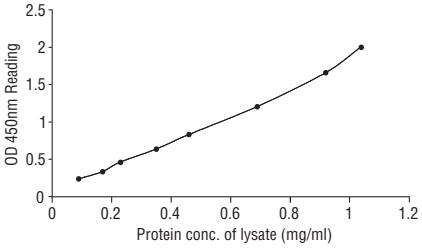

Figure 2: Linear relationship between protein concentration of lysates from UV-treated 293 cells and kit assay optical density readings. 293 cells (50-70% confluence) were treated with UV light and lysed after growth at 37ºC for 30 min.

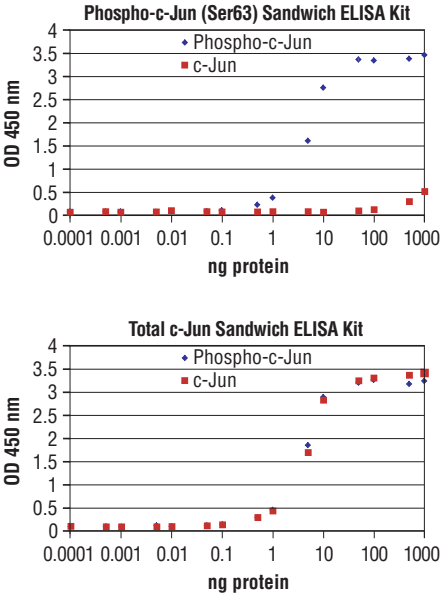

Figure 3: Recombinant c-Jun protein #6093 was incubated with and without purified SAPK/JNK in an in vitro kinase reaction. After the reaction, phosphorylated c-Jun was detected and measured using the PathScan® Phospho-c-Jun (Ser 63) Sandwich ELISA kit #7260 (upper), while non-phosphorylated c-Jun was not detected. Both phosphorylated and non-phosphorylated c-Jun were detected using PathScan Total c-Jun Sandwich ELISA kit #7270 (lower). The linear range for the recombinant c-Jun protein is between 1-10 ng for both Sandwich ELISA kits.

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Sandwich ELISA Protocol

A Reagent Preparation

A1 Bring all microwell strips to room temperature before use.

A2 Prepare 1X Wash Buffer using Milli-Q or equivalently purified water.

A3 **1X Cell Lysis Buffer from CST #9803:** 20 mM Tris (pH7.5), 150 mM NaCl, 1 mM ethylene diaminetetraacetate (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-glycerolphosphate, 1 mM Na_3VO_4, 1µg/ml leupeptin. This buffer can be stored at 4°C for short-term use (1–2 weeks).

B Preparing Cell Lysates

B1 Aspirate media. Treat cells by adding fresh media containing regulator for desired time.

B2 To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.

B3 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.

B4 Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.

B5 Sonicate lysates on ice.

B6 Microcentrifuge for 10 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Aliquot and store at –80°C.

C Test Procedure

C1 After the microwell strips have reached room temperature, break off the required numbered of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.

C2 Add 100 µl of **Sample Diluent** (blue color) to a microcentrifuge tube. Transfer 100 µl of cell lysate into the tube and vortex for a few seconds. (Sample applied to the well can be diluted 1:1 when the suggested cell lysis buffer is used for cell extraction.) Figure 2 provides a reference in dilution factor for lysates and kit assay results.

C3 Add 100 µl of each diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hours at 37°C. Alternatively, the plate can be incubated overnight at 4°C, which gives the best detection of target protein.

C4 Gently remove the tape and wash wells:

a. Discard plate contents into a receptacle.

b. Wash 4 times with 1X Wash Buffer, 200 µl each time for each well.

c. 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.

d. Clean the underside of all wells with a lint-free tissue.

C5 Add 100 µl of **Detection Antibody** (green color) to each well. Seal with tape and incubate the plate for 1 hour at 37°C.

C6 Repeat wash procedure as in Step C4.

C7 Add 100 µl of **HRP-Linked** secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 minutes at 37°C.

C8 Repeat wash procedure as in Step C4.

C9 Add 100 µl of **TMB Substrate** to each well. Seal with tape and incubate the plate for 10 minutes at 37°C or 30 minutes at 25°C.

C10 Add 100 µl of **STOP Solution** to each well. Shake gently for a few seconds.

Note: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.

C11 Read results.

a. Visual Determination — Read within 30 minutes after adding STOP Solution.

b. Spectrophometric Determination — Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 minutes after adding STOP Solution.