

PathScan® Total SAPK/JNK Sandwich ELISA Kit

✓ 1 Kit
(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 #5599
Swiss-Prot Acc. #P45983

Species Cross-Reactivity: H, M

Description: CST's PathScan® Total SAPK/JNK Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total SAPK/JNK protein. A SAPK/JNK (7325-7E7) Mouse mAb* has been coated onto the microwells. After incubation with cell lysates, Both nonphospho- and phospho-SAPK/JNK proteins are captured by the coated antibody. Following extensive washing, a SAPK/JNK (56G8) Rabbit mAb #9258* is added to detect the captured SAPK/JNK protein. HRP-linked anti-rabbit antibody #7074* 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 total SAPK/JNK protein.

* Antibodies in this kit are custom formulations specific to the kit.

Specificity/Sensitivity: CST's PathScan® Total SAPK/JNK Sandwich ELISA Kit detects endogenous levels of total SAPK/JNK protein. Using PathScan® Phospho-SAPK/JNK (Thr183/Tyr185) Sandwich ELISA Kit #7325, a significant induction of phospho-SAPK/JNK in 293 cells treated with UV can be detected. However, the level of total SAPK/JNK (phospho and nonphospho), detected by this Sandwich ELISA Kit #7330, remains unchanged (Figure 1).

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

Background: The stress-activated protein kinase/Jun-amino-terminal kinase SAPK/JNK is potently and preferentially activated by a variety of environmental stresses including UV and gamma radiation, ceramides, inflammatory cytokines and in some instances, by growth factors and GPCR agonists (1-6). As with the other MAPKs, the core signaling unit is composed of a MAPKKK, typically MEKK1-MEKK4, or by one of the mixed lineage kinases (MLKs), which phosphorylate and activate MKK4/7. Upon activation, MKKs phosphorylate and activate the SAPK/JNK kinase (2). Stress signals are delivered to this cascade by small GTPases of the Rho family (Rac, Rho, cdc42) (3). Both Rac1 and cdc42 mediate the stimulation of MEKs and MLKs (3). Alternatively, MKK4/7 can be activated in a GTPase independent mechanism via stimulation of a germinal center kinase (GCK) family member (4). There are three SAPK/JNK genes each of which undergoes alternative splicing resulting in numerous isoforms (3). SAPK/JNK, when active as a dimer, can translocate to the nucleus and regulate transcription through its effects on c-Jun, ATF-2 and other transcription factors (3,5).

Products Included	Volume	Solution Color
SAPK/JNK Mouse Antibody Coated Microwells*	96 tests	
SAPK/JNK Detection Antibody	11 ml	green
Anti-rabbit IgG, HRP-linked Antibody	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 with the exception of 10X Cell Lysis Buffer, which is stored at -20°C (packaged separately).

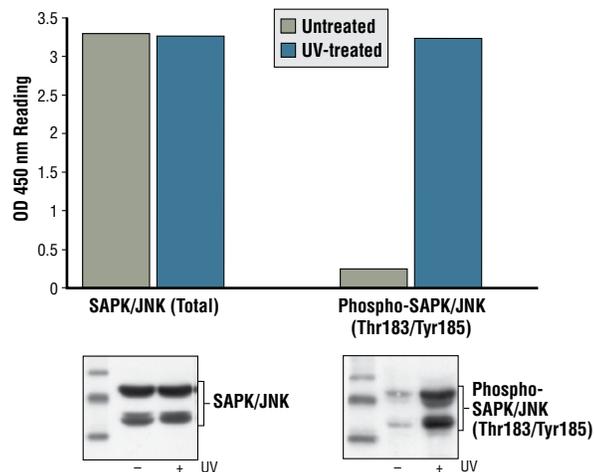


Figure 1: Treatment of 293 cells with UV stimulates phosphorylation of SAPK/JNK at Thr183/Tyr185, detected by PathScan® Phospho-SAPK/JNK (Thr183/Tyr185) Sandwich ELISA kit, #7325, but does not affect the level of total SAPK/JNK protein detected by this PathScan® Total SAPK/JNK Sandwich ELISA kit, #7330. OD450 readings are shown in the top figure, while the corresponding western blot using Phospho-SAPK/JNK (Thr183/Tyr185) Antibody #9251 (right panel) or SAPK/JNK Rabbit mAb #9258 (left panel), is shown in the bottom figure.

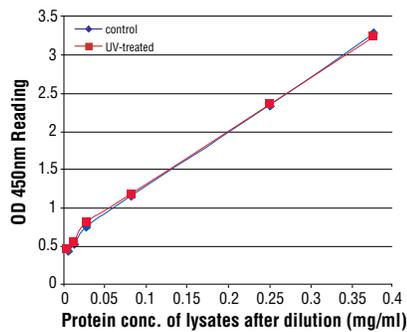


Figure 2: Linear relationship between protein concentration of lysates from control and UV-treated 293 cells and kit assay optical density readings. 293 cells (80% confluence) were treated with UV and lysed after incubation at 37°C for 30 minutes.

Background References:

- (1) Davis, R.J. (1999) *Biochem Soc Symp* 64, 1-12.
- (2) Ichijo, H. (1999) *Oncogene* 18, 6087-93.
- (3) Kyriakis, J.M. and Avruch, J. (2001) *Physiol Rev* 81, 807-69.
- (4) Kyriakis, J.M. (1999) *J Biol Chem* 274, 5259-62.
- (5) Leppä, S. and Bohmann, D. (1999) *Oncogene* 18, 6158-62.
- (6) Whitmarsh, A.J. and Davis, R.J. (1998) *Trends Biochem Sci* 23, 481-5.

Sandwich ELISA Protocol

A Reagent Preparation

1. Bring all microwell strips to room temperature before use.
2. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in Milli-Q or equivalently purified water.
3. **1X Cell Lysis Buffer from CST #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. This buffer can be stored at 4°C for short-term use (1–2 weeks).

B 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 phenyl-methylsulfonyl fluoride (PMSF) to each plate (10 cm in diameter) 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.

C Test Procedure

1. After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.
2. Add 100 μl of Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color) to a microcentrifuge tube. Transfer 100 μl of cell lysate into the tube and vortex for a few seconds. Generally, sample applied to the well can be diluted 1:1 when the suggested cell lysis buffer is used for cell extraction. Individual data sheets for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results. However, dilution factors need to be titrated when specific cell lysates are used.

3. 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.
4. 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.
5. Add 100 μl of Detection Antibody (green color) to each well. Seal with tape and incubate the plate for 1 hour at 37°C.
6. Repeat wash procedure as in Step 4.
7. 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.
8. Repeat wash procedure as in Step 4.
9. 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.
10. 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.

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