

PathScan® Phospho-SAPK/JNK (Thr183/Tyr185) Sandwich ELISA Antibody Pair

✓ 1 Kit
(4 X 96 assays)



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

Species Cross-Reactivity: H, M

Description: CST's PathScan® Phospho-SAPK/JNK (Thr183/Tyr185) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-SAPK/JNK (Thr183/Tyr185) Sandwich ELISA Kit #7325. Capture and Detection antibodies (100X stocks) and HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The Phospho-SAPK/JNK (Thr183/Tyr185) Capture Antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysates are added followed by SAPK/JNK Detection Antibody and Anti-mouse IgG, HRP-conjugated Antibody. HRP substrate, TMB, is added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of Phospho-SAPK/JNK (Thr183/Tyr185) protein.

Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: For Antibody Pair specificity and sensitivity, please refer to the corresponding PathScan® Sandwich ELISA Kit. Note: This antibody pair detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

** 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 the included protocol. Solutions should be made fresh daily.

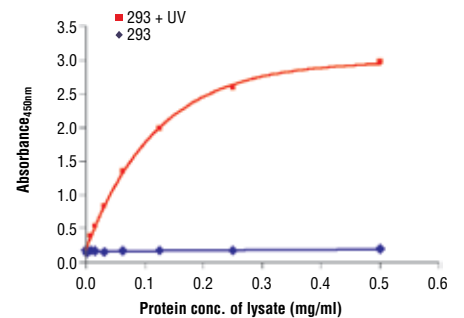
Products Included	Item #	Volume	Cap Color	Storage
Phospho-SAPK/JNK (Thr183/Y185) Capture Rabbit mAb (100X)	32090	400 µL	Pink	4°C
SAPK/JNK Detection Mouse mAb (100X)	16945	400 µL	Blue	4°C
Anti-mouse IgG, HRP-linked Antibody (1000X)	16736	40 µL	Yellow	-20°C

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

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, 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 MEKKs 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).

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.



The relationship between lysate protein concentration from untreated and UV-treated 293 cells and the absorbance at 450 nm using PathScan® Phospho-SAPK/JNK (Thr183/Tyr185) Sandwich ELISA Antibody Pair #7217. 293 cells were treated with UV, allowed to recover for 30 minutes at 37°C and then lysed.

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.