

PathScan® Phospho-p38 α MAPK (Thr180/Tyr182) Sandwich ELISA Kit

✓ 1 Kit
(96 assays)



Cell Signaling
TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignaling.com

Support ■ 877-678-TECH (8324)
info@cellsignaling.com

Web ■ www.cellsignaling.com

rev. 05/16/07

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Species Cross-Reactivity: H, M, R

Introduction: CST's PathScan® Phospho-p38 α MAP kinase (Thr180/Tyr182) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of phospho-p38 α MAP kinase (Thr180/Tyr182) protein. A p38 α MAP kinase antibody (#9218*) has been coated onto the microwells. After incubation with cell lysates, p38 α MAP kinase protein is captured by the coated antibody. Following extensive washing, Phospho-p38 MAPK (Thr180/Tyr182) (28B10) monoclonal antibody (#9216*) is added to detect the captured phospho-p38 α MAP kinase 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-p38 α MAP kinase (Thr180/Tyr182) protein.

* Antibodies in kit are custom formulations specific to kit.

Companion Products:

Phospho-p38 MAPK (Thr180/Tyr182) (28B10) Mouse mAb #9216

p38 α MAP Kinase Antibody #9218

Anti-mouse IgG, HRP-linked Antibody #7076

Specificity/Sensitivity: CST's PathScan® Phospho-p38 α MAP kinase (Thr180/Tyr182) Sandwich ELISA Kit detects endogenous levels of phospho-p38 α MAP kinase (Thr180/Tyr182) protein. Using this Sandwich ELISA Kit #7140, a significant induction of phospho-p38 α MAP kinase (Thr180/Tyr182) in NIH/3T3 cells treated with UV light is detected. However, the level of total p38 α MAP kinase remains unchanged, as shown by Western analysis using p38 α MAP kinase Antibody (#9218) (figure 1). Both C6 and 293 cells treated either UV light or anisomycin show similar results (data not shown).

Products Included	Volume	Solution Color
p38 MAPK α Ab Coated Microwells*	96 tests	
p38 MAPK (28B10) Monoclonal Detection Ab	11 ml	green
Anti-mouse 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).

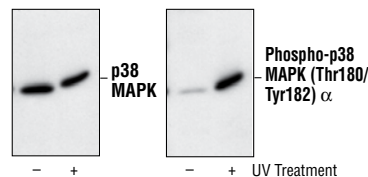
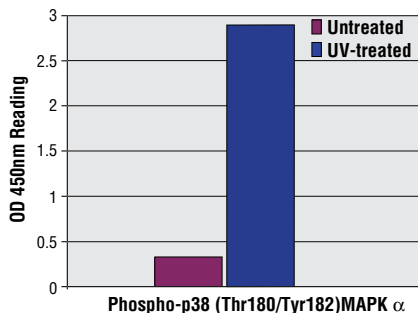


Figure 1: Treatment of NIH/3T3 cells with UV light stimulates phosphorylation of p38 α MAP kinase at Thr180/Tyr182, detected by PathScan® Phospho-p38 α MAP kinase α (Thr180/Tyr182) Sandwich ELISA kit, #7140, but does not affect the level of total p38 α MAP kinase protein detected by p38 α MAP kinase Antibody (#9218) via Western analysis. OD 450 readings are shown in the top figure, while the corresponding Western blot using Phospho-p38 MAP kinase (Thr180/Tyr182) (28B10) mAb #9216 or p38 α MAP kinase Antibody #9218, is shown in the bottom figure.



Background: p38 MAP kinase (MAPK), also called RK (1) or CSBP (2), is the mammalian orthologue of the yeast HOG kinase which participates in a signaling cascade controlling cellular responses to cytokines and stress (1-4). Four isoforms of p38 MAP kinase, p38 α , β , γ (also known as ERK6 or SAPK3) and δ (also known as SAPK4) have been identified. Similar to the SAPK/JNK pathway, p38 MAP kinase is activated by a variety of cellular stresses including osmotic shock, inflammatory cytokines, lipopolysaccharides (LPS), UV light and growth factors (1-5). MKK3, MKK6 and SEK activate p38 MAP kinase by phosphorylation at Thr180 and Tyr182. Activated p38 MAP kinase has been shown to phosphorylate and activate MAPKAP kinase 2 (3) and to phosphorylate the transcription factors ATF-2 (5), Max (6) and MEF2 (5-8).

Background References:

- (1) Rouse, J. et al. (1994) *Cell* 78, 1027–1037.
- (2) Han, J. et al. (1994) *Science* 265, 808–811.
- (3) Lee, J.C. et al. (1994) *Nature* 372, 739–746.
- (4) Freshney, N.W. et al. (1994) *Cell* 78, 1039–1049.
- (5) Raingeaud, J. et al. (1995) *J. Biol. Chem.* 270, 7420–7426.
- (6) Zervos, A.S. et al. (1995) *Proc. Natl. Acad. Sci. USA* 92, 10531–10534.
- (7) Zhao, M. et al. (1999) *Mol. Cell. Biol.* 19, 21–30.
- (8) Yang, S.H. et al. (1999) *Mol. Cell. Biol.* 19, 4028–4038.

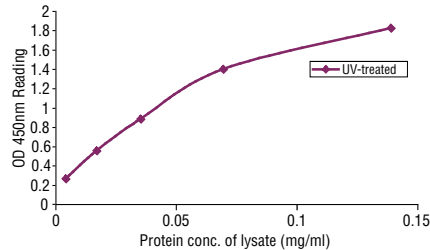


Figure 2: Linear relationship between protein concentration and kit assay optical density readings, using UV-treated NIH/3T3 cell lysates. NIH/3T3 cells (50–70% confluence) were treated with UV light and lysed after growth at 37°C for 30 min.

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.