

# PathScan® MAP Kinase Multi-Target Sandwich ELISA Kit

✓ 1 Kit  
(96 assays)

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

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

**Web** ■ www.cellsignal.com

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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.

## Species Cross-Reactivity: H, M

**Introduction:** CST's PathScan® MAP Kinase Multi-Target Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that combines the reagents necessary to detect endogenous levels of phospho-p44/42 MAPK (Thr202/Tyr204), phospho-p38 MAPK (Thr180/Tyr182), MEK1, phospho-MEK1 (Ser217/221), SAPK/JNK and phospho-SAPK/JNK (Thr183/Tyr185). These molecules represent convergence points and key regulatory proteins in signaling pathways controlling cellular events such as growth, differentiation and the response to stress and inflammation. Sixteen tests are provided for each target protein. Specific assay formulations for the indicated target proteins can be found in the datasheets associated with the individual PathScan® Sandwich ELISA Kits\*\*. Briefly, a capture antibody\* has been coated onto the microwells. After incubation with cell lysates, the target protein is captured by the coated antibody. Following extensive washing, a detection antibody\* is added to detect the captured target protein. An HRP-linked secondary antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of absorbance for this developed color is proportional to the quantity of bound target protein.

\*Antibodies in kit are custom formulations specific to kit.

\*\*See companion products.

**Specificity/Sensitivity:** CST's PathScan® MAP Kinase Multi-Target Sandwich ELISA Kit #7274 detects endogenous levels of six proteins: phospho-p44/42 MAPK (Thr202/Tyr204), phospho-p38 MAPK (Thr180/Tyr182), MEK1, phospho-MEK1 (Ser217/221), SAPK/JNK and phospho-SAPK/JNK (Thr183/Tyr185). Differential phosphorylation of these proteins can be observed over time in response to various growth factor and cytokine treatments. As shown in Figure 1. The relationship between the protein concentration of the lysate and the absorbance at 450 nm can be found in the datasheets associated with the individual PathScan® Sandwich ELISA Kits\*\*.

\*\*See companion products.

## Companion Products:

PathScan® Phospho-p38 MAPK (Thr180/Tyr182) Sandwich ELISA Kit #7946

PathScan® Total MEK1 Sandwich ELISA Kit #7165

PathScan® Phospho-MEK1 (Ser217/221) Sandwich ELISA Kit #7175

PathScan® Total SAPK/JNK Sandwich ELISA Kit #7330

PathScan® Phospho-SAPK/JNK (Thr183/Tyr185) Sandwich ELISA Kit #7325

PathScan® Phospho-p44/42 MAPK (Thr202/Tyr204)

Products Included	Quantity	Solution Color	Cap Color
Phospho-p44/42 MAPK (Thr202/Tyr204) Rabbit Antibody Coated Microwells	16 tests		
p44/42 MAPK Mouse Detection Antibody	1.8 ml	Green	Light Pink
Anti-Mouse IgG HRP-Linked Antibody	1.8 ml	Red	Light Pink
p38 MAP Kinase Mouse Antibody Coated Microwells	16 tests		
Phospho-p38 MAPK (Thr180/Tyr182) Rabbit Detection Antibody	1.8 ml	Green	Yellow
Anti-Rabbit IgG HRP-Linked Antibody	1.8 ml	Red	Yellow
MEK1 Mouse Antibody Coated Microwells	16 tests		
MEK1 Rabbit Detection Antibody	1.8 ml	Green	White
Anti-Rabbit IgG HRP-Linked Antibody	1.8 ml	Red	White
MEK1 Mouse Antibody Coated Microwells	16 tests		
Phospho-MEK1/2 (Ser217/221) Rabbit Detection Antibody	1.8 ml	Green	Green
Anti-Rabbit IgG HRP-Linked Antibody	1.8 ml	Red	Green
SAPK/JNK Mouse Antibody Coated Microwells	16 tests		
SAPK/JNK Rabbit Detection Antibody	1.8 ml	Green	Gray
Anti-Rabbit IgG HRP-Linked Antibody	1.8 ml	Red	Gray
SAPK/JNK Mouse Antibody Coated Microwells	16 tests		
Phospho-SAPK/JNK (Thr183/Tyr185) Rabbit Detection Antibody	1.8 ml	Green	Purple
Anti-rabbit IgG HRP-Linked Antibody	1.8 ml	Red	Purple
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	
Cell Lysis Buffer (10X)	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).

Sandwich ELISA Kit #7177

Anti-rabbit IgG, HRP-linked Antibody #7074

Anti-mouse IgG, HRP-linked Antibody #7076

Cell Lysis Buffer (10X) #9803

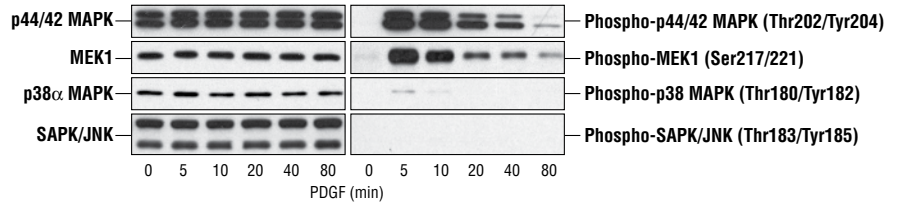
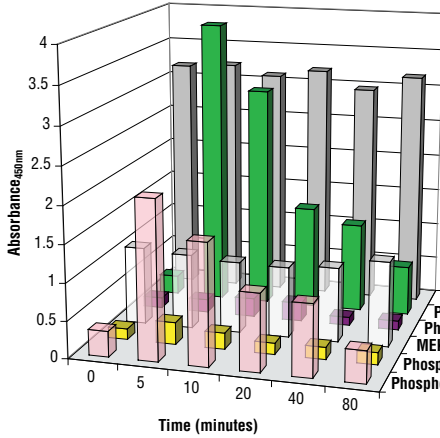
TMB Substrate #7004

STOP Solution #7002

Phosphate Buffered Saline (PBS-20X) #9808

Phosphate Buffered Saline with Tween 20 (PBST-20X) #9809

**A. PDGF**



**B. TNF-α/IL-1β**

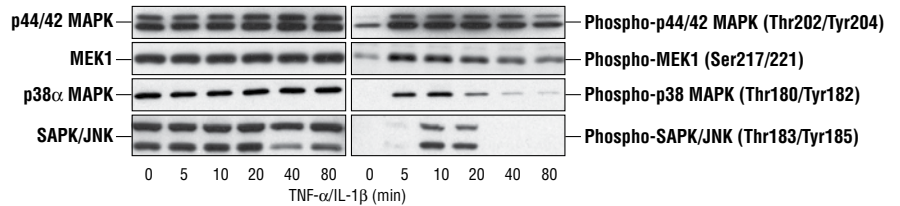
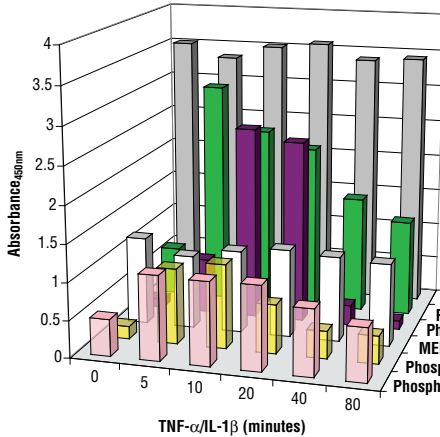


Figure 1. Treatment of NIH/3T3 cells with PDGF (A) or TNF-α/IL-1β (B) induces differential phosphorylation of p44/42 MAPK at Thr202/Tyr204, MEK1 at S217/221, p38α MAPK at Thr180/Tyr182, and SAPK/JNK at Thr183/Tyr185 as detected by the PathScan® MAP Kinase Multi-Target Sandwich ELISA Kit #7274. While dynamic phosphorylation is observed throughout the time course, the level of total p44/42 MAPK, MEK1, p38 MAPK and SAPK/JNK remains unchanged as demonstrated by sandwich ELISA and Western analysis. NIH/3T3 cells (80-90% confluent) were starved overnight and stimulated with PDGF (100 ng/mL) for 5, 10, 20, 40 and 80 minutes at 37°C. For simultaneous treatment with TNF-α and IL-1β, exponentially growing cultures of NIH/3T3 (80-90% confluent) were treated for the indicated times at 37°C with 20 ng/mL TNF-α and 10 ng/mL IL-1β. Lysates were assayed at a protein concentration of 0.5 mg/mL. The absorbance readings at 450 nm are shown as a 3-dimensional representation in the left figure, while the corresponding Western blots are shown in the right figure. The antibodies used for the Western analyses include p44/42 MAP Kinase Antibody #9102, Phospho-p44/42 MAPK (Thr202/Tyr204) (E10) Mouse mAb #9106, MEK1 (61B12) Mouse mAb #2352, Phospho-MEK1/2 (Ser217/221) Antibody #9121, p38α MAP Kinase Antibody #9218, Phospho-p38 MAPK (Thr180/Tyr182) (28B10) Mouse mAb #9216, SAPK/JNK (56G8) Rabbit mAb #9258 and Phospho-SAPK/JNK (Thr183/Tyr185) (G9) Mouse mAb #9255.

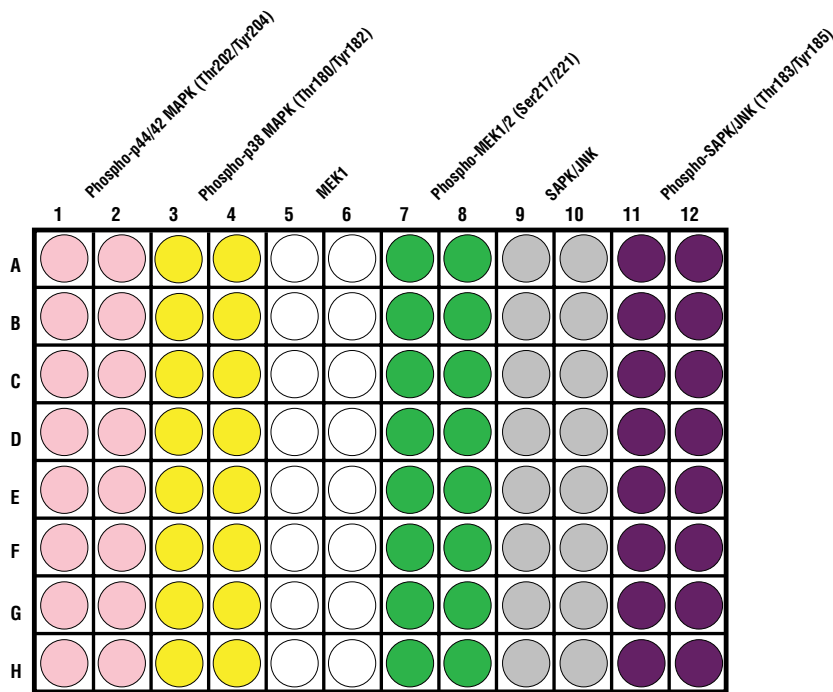


Figure 2. Schematic representation of a 96-well plate depicting the color-code of the reagents used to detect endogenous levels of phospho-p44/42 MAPK (Thr202/Tyr204) (light pink; 1 & 2), Phospho-p38 (Thr180/Tyr182) (yellow; 3 & 4), MEK1 (white; 5 & 6), Phospho-MEK1 (Ser217/221) (green; 7 & 8), SAPK/JNK (gray; 9 & 10) and Phospho-SAPK/JNK (Thr183/Tyr185) (purple; 11 & 12).

**Background:** Both p44 and p42 MAP kinases (Erk1 and Erk2) function in a protein kinase cascade that plays a critical role in the regulation of cell growth and differentiation (1-6). MAP kinases are activated by a wide variety of extracellular signals including growth and neurotrophic factors, cytokines, hormones and neurotransmitters. Activation of MAP kinases occurs through phosphorylation of threonine and tyrosine (Thr202 and Tyr204 of human MAP kinase or Thr183 and Tyr185 of rat MAP kinase) at the sequence T\*EY\* by a single upstream MAP kinase kinase (MEK) (7,8).

MEK1 and MEK2 are dual-specificity protein kinases that function in a mitogen activated protein kinase cascade controlling cell growth and differentiation. Activation of MEK1 and MEK2 occurs through phosphorylation of Ser217 and Ser221 by Raf-like molecules. MEK activates p44 and p42 MAP kinase (1,9,10).

p38 MAP kinase (MAPK) participates in a signaling cascade controlling the cellular response to pro-inflammatory cytokines and a variety of cellular stresses. MKK3, MKK6 and SEK (MKK4) activate p38 MAP kinase by phosphorylation at Thr180 and Tyr182 (11-14).

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The stress-activated protein kinase/Jun-amino-terminal kinase SAPK/JNK is 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 (15-20). As with the other MAPKs, the core-signaling unit is composed of a MAPKKK, typically MEKK1-4, or by a mixed lineage kinase (MLK), which phosphorylates and activates MKK4-7, which then phosphorylates Thr183 and Tyr185 to activate the SAPK/JNK kinase (16). Stress signals are delivered to this cascade by small GTPases of the Rho family (Rac, Rho, cdc42) (17). Both Rac1 and cdc42 mediate the stimulation of MEKs and MLKs (17). Alternatively, MKK4-7 can be activated by a pathway independent of small GTPases via stimulation of a member of the germinal center kinase (GCK) family (18).

#### Background References:

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## 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 Na<sub>3</sub>VO<sub>4</sub>, 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-colored solution) to each well. **Be sure to match the cap color of the Detection Antibody with the corresponding color code of the 8-well strip.** 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-colored solution) to each well. **Be sure to match the cap color of the Detection Antibody with the corresponding color code of the 8-well strip.** 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.