

# PathScan® Total MEK1 Sandwich ELISA Kit

✓ 1 Kit  
(96 assays)



Cell Signaling  
TECHNOLOGY®

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This product is for *in vitro* research use only and is not intended for use in humans or animals.  
This product is not intended for use as a therapeutic or in diagnostic procedures.

## Species Cross-Reactivity: H, M

**Introduction:** CST's PathScan® Total MEK1 Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total MEK1 protein. MEK1 (61B12) Mouse mAb #2352\* has been coated onto the microwells. After incubation with cell lysates, total MEK1 protein (phospho- and nonphospho-) is captured by the coated antibody. Following extensive washing, MEK1/2 Antibody #9122\* is added to detect the captured MEK1 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 MEK1 protein.

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

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

**Specificity/Sensitivity:** CST's PathScan® Total MEK1 Sandwich ELISA Kit detects endogenous levels of total MEK1 protein. A significant induction of MEK1 phosphorylation can be detected in PMA-treated NIH/3T3 cells using PathScan® Phospho- MEK1 (Ser217/221) Sandwich ELISA Kit #7175. However, the level of total MEK1 detected by this sandwich ELISA kit #7165 remains unchanged (Figure 1). This kit can also be used to detect total MEK1 protein in human 293 cells.

**Background:** MEK1 and MEK2, also called MAPK or Erk kinases, are dual-specificity protein kinases that function in a mitogen activated protein kinase cascade controlling cell growth and differentiation (1-3). Activation of MEK1 and MEK2 occurs through phosphorylation of two serine residues at positions 217 and 221 (in the activation loop of subdomain VIII) by Raf-like molecules. MEK1/2 is activated by a wide variety of growth factors and cytokines and also by membrane depolarization and calcium influx (1-4). Constitutively active forms of MEK1/2 are sufficient for the transformation of NIH/3T3 cells or the differentiation of PC12 cells (4). MEK activates p44 and p42 MAP kinase by phosphorylating both threonine and tyrosine residues at sites located within the activation loop of kinase subdomain VIII.

## Background References:

- Crews, C.M. et al. (1992) *Science* 258, 478-480.
- Alessi, D.R. et al. (1994) *EMBO J.* 13, 1610-1619.
- Rosen, L.B. et al. (1994) *Neuron* 12, 1207-1221.
- Cowley, S. et al. (1994) *Cell* 77, 841-852.

Products Included	Volume	Solution Color
MEK1 (61B12) Mouse mAb Coated Microwells *	96 tests	
MEK1/2 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).

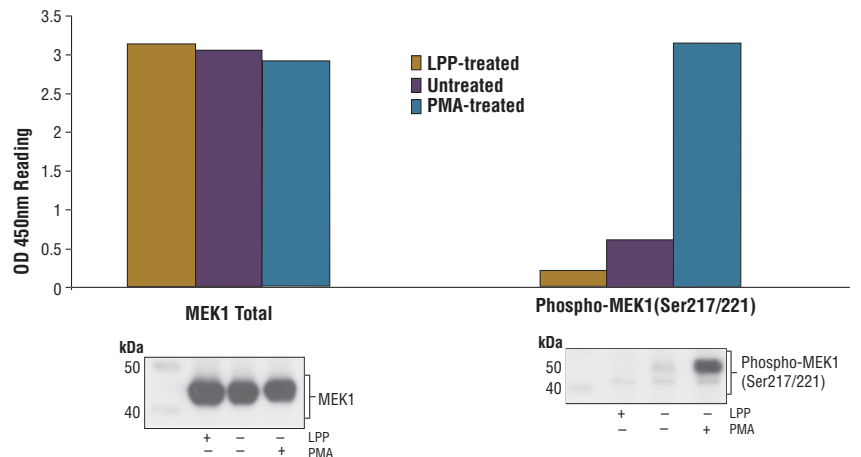
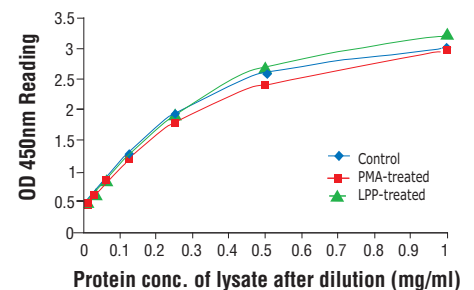


Figure 1: Treatment of NIH/3T3 cells with PMA stimulates phosphorylation of MEK1 at Ser217/221, detected by PathScan® Phospho-MEK1 (Ser217/221) Sandwich ELISA kit #7175, but does not affect the level of total MEK1 protein detected by PathScan® Total MEK1 Sandwich ELISA kit #7165.  $\lambda$  protein phosphatase (LPP) treatment of control cell lysates (37°C for 90 minutes) abolished the basal phosphorylation of MEK1 in control lysates shown in both Sandwich ELISA and Western analysis. The OD<sub>450</sub> readings are shown in the top figure, while the corresponding Western blot, using Phospho-MEK1/2 (Ser217/221) Antibody #9121 (right panel) or MEK1 Mouse mAb #2352 (left panel), is shown in the bottom figure.

Figure 2: Linear relationship between protein concentration of lysates from untreated and PMA-treated NIH/3T3 cells and kit assay optical density readings. Cells (80% confluence) were treated with PMA (120 ng/ml) and lysed after incubation at 37°C for 30 minutes.  $\lambda$  protein phosphatase (LPP) treatment of control cell lysates was performed at 37°C for 90 minutes.



**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine

Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

## 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 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.