

PathScan® Total MEK1 Sandwich ELISA Antibody Pair

✓ 1 Kit
(4 X 96 assays)

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New 12/07

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

Entrez-Gene ID #1432
Swiss-Prot Acc. #Q16539

Species Cross-Reactivity: H, M

Description: CST's PathScan® Total MEK1 Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Total MEK1 Sandwich ELISA Kit #7165. 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 MEK1 Capture Antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysates are added followed by a MEK1/2 Detection Antibody and anti-Rabbit 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 total MEK1/2 protein.

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

Reagents not supplied:

Phosphate Buffered Saline (PBS-20X) #9808

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

Cell Lysis Buffer (10X) #9803

TMB Substrate #7004

STOP Solution #7002

Blocking Buffer: 1X PBS/0.05% Tween-20, 1% BSA

96 Well Microplates**

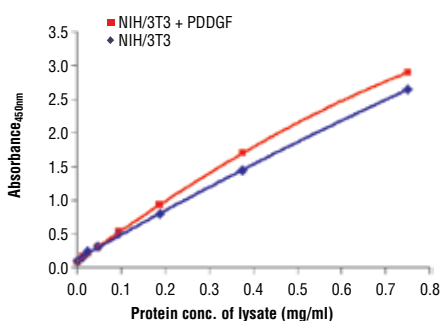
Microplate Reader

** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592) and Corning® 96 Well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene High Bind Microplates (#3369).

Note: Antibody pairs have been optimized using recommended buffers, reagents, plates and the included protocol. Solutions should be made fresh daily.

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).

Products Included	Volume	Cap Color	Storage
Total MEK1 Capture Antibody (100X)	0.4 ml	Pink	4°C
Total MEK1/2 Detection Antibody (100X)	0.4 ml	Blue	4°C
Anti-Rabbit IgG, HRP-Linked Antibody (1000X)	0.04 ml	Red	-20°C



The relationship between lysate protein concentration from untreated and PDGF-treated NIH/3T3 cells and the absorbance at 450 nm using PathScan® Total MEK1 Sandwich ELISA Antibody Pair #7215 is shown. NIH/3T3 cells were treated with PDGF for 20 minutes at 37°C and then lysed.

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:

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

Storage: MEK1 Capture and MEK1/2 Detection Antibodies are stored at 4°C. Anti-Rabbit IgG, HRP-Linked Antibody is stored at -20°C.

Companion Products:

Phosphate Buffered Saline (PBS-20X) #9808

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

BSA #9998

TMB Substrate #7004

STOP Solution #7002

PathScan® Total MEK1 Sandwich ELISA Kit #7165

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

PathScan® Phospho-MEK1 (Ser217/221) Sandwich ELISA Antibody Pair #7211

Anti-rabbit IgG, HRP-linked Antibody #7074

MEK1 (61B12) Mouse mAb #2352

MEK1/2 Antibody #9122

MEK1 (30C8) Rabbit mAb #9146

MEK1/2 (47E6) Rabbit mAb #9126

MEK1/2 (L38C12) Mouse mAb #4694

Cell Lysis Buffer (10X) #9803

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.