

Anti-phospho-MEK1 (Ser218/222)/MEK2 (Ser222/226)

(rabbit monoclonal IgG)

Catalog # 05-747

Lot # 26733

Immunogen: KLH-conjugated, synthetic peptide containing ...DpSMANpSF... in which pS corresponds to phosphorylated amino acids 218 and 222 of human MEK1. The immunizing sequence is highly conserved.

Specificity: Recognizes phosphorylated MEK1/MEK2, Mr ~45kDa.

Species Cross-reactivity: Human, mouse and rat.

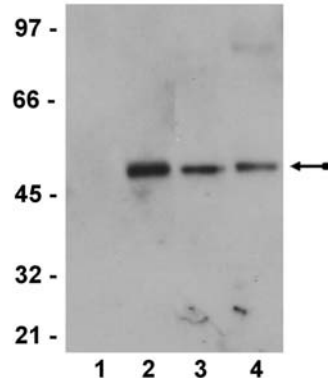
Formulation: 100µl of protein A purified rabbit IgG in 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 1:2,000-1:10,000 of this lot detected phosphorylated MEK1/2 in RIPA lysates from PC-12 cells that had been treated with 5 Units of B-Raf for 30 minutes at 30°C.



Immunoblot Analysis

Untreated (Lanes 1 and 3) and B-Raf phosphorylated (Lanes 2 and 4) PC-12 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-MEK1 (Ser218/222)/MEK2 (Ser222/226) (1:5000, Lanes 1 and 2) or total anti-MEK1 (1:1000, Catalog # 06-269, Lanes 3 and 4). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates MEK1 (~45kDa).

General References:

1. Hodge, C., *et al.*, *J. Biol. Chem.* **273**: 31327-31336, 1998.
2. Gopalbhai, K., *et al.*, *J. Biol. Chem.* **278**: 8118-8125, 2003.

Produced in collaboration with Epitomics, Inc.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1 μ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 3% nonfat dry milk (Catalog # 20-200) in TBS with 0.05% Tween 20 (TBST-MLK) for 30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with a **1:2,000-1:10,000 dilution of anti-phospho-MEK1 (Ser218/222)/MEK2 (Ser222/226)**, diluted in freshly prepared TBST-MLK for 1.25 hours with agitation at room temperature.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in TBST-MLK for 0.5 hours at room temperature with agitation.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).