

## Certificate of Analysis

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

(rabbit monoclonal IgG)

Catalog # 05-747

Lot # 30004

**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 70% storage buffer (PBS, 0.05% sodium azide) and 30% glycerol. Store at -20°C.

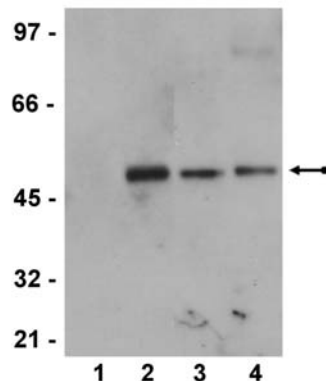
**Storage and Stability:** Stable for 2 years at -20°C from date of shipment.

**Handling Recommendations:** Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.** Note: Variability in freezer temperatures below -20°C may cause glycerol-containing solutions to become frozen during storage.

**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

Representative blot from a previous lot. 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.

### 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 EDTA; 1mM PMSF; 1 $\mu$ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 3% nonfat dry milk (Catalog # 20-200) and 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 2.5 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).