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## Certificate of Analysis

### Anti-rabbit IgG, HRP-conjugate

Catalog #12-348

Lot # 21472

**Immunogen:** Highly purified whole rabbit IgG.

**Specificity:** Specific for rabbit IgG heavy and light chains.

**Species Cross-reactivity:** Rabbit

**Formulation:** 500mg of goat IgG, conjugated with horseradish peroxidase, in 500ml 0.02M potassium phosphate, 0.15M NaCl, pH 7.2, 10mg/ml BSA, 0.01% (w/v) gentamicin sulfate with glycerol added to 50%. Liquid at -20°C.

**Storage and Stability:** Stable for 1 year at -20°C from date of shipment.

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

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### Quality Control Testing

**Immunoblot Analysis:** This lot of antibody was tested at 1:1000, 1:2000, 1:3000, 1:4000, 1:5000 and 1:10,000 dilutions in conjunction with a rabbit polyclonal anti-MAP Kinase 1/2 (Erk 1/2-CT), Catalog # 06-182, antibody to detect MAPK (42-44kDa) in a mouse 3T3 cell lysate.

The recommended dilution for this lot is 1:5000.

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### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1µg/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 PBS containing 3% nonfat dry milk (PBS-MLK) for 45 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **a rabbit primary antibody**, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in **1:1000-1:10,000 goat anti-rabbit IgG, HRP-conjugated** in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-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).