

Certificate of Analysis

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Anti-Eck/EphA2, clone D7(mouse monoclonal IgG₁)

Catalog # 05-480

Lot # DAM1400115

Immunogen: Native protein isolated by purification of phosphotyrosine-containing proteins. Clone D7.

Specificity: Recognizes Eck/EphA2, Mr 140kDa.

Species Cross-reactivity: Human, mouse, rat, bovine and canine.

Formulation: 200µg of protein G purified mouse IgG₁ in 200µl of 70% storage buffer (0.2M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide) and 30% glycerol. Store at -20°C.

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

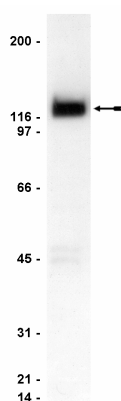
Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.** Note: Variability in freezer temperatures 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: 0.5-2µg/ml of this lot detected Eck in RIPA lysates from human A431 and previously from foreskin fibroblasts, murine 3T3/A31 and rat L6 cells.

Included Positive Antigen Control: Catalog # 12-301, non stimulated A431 lysate. **Add 2.5µl of 2-mercaptoethanol/100µl of lysate and boil for 5 minutes to reduce the preparation.** Load 20µg of reduced lysate per lane for minigels.

**Immunoblot Analysis**

Representative blot from a previous lot. A431 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Eck/EphA2 (0.5µg/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Eck (~140kDa).

Additional Research Applications

Protein Kinase Assay: This antibody has been reported to have been used in an immunoprecipitation autophosphorylation assay, using a Mn-PIPES reaction buffer.¹

Immunoprecipitation: This antibody has been reported to immunoprecipitate Eck from 500µg of a human breast epithelial cell line which had been lysed in TBS containing 1% Triton X-100. Use 1-4 µg per reaction.

Immunocytochemistry: This antibody has been reported to immunostain Eck in human, mouse and rat epithelial cells fixed with 3.7% formaldehyde solution and permeabilized with 0.5% Triton X-100 in TBS.

General References:

1. Romer, L., *et al.*, *Mol. Biol. Cell* **5**: 349-361, 1994.
2. Magal, E., *et al.*, *J. Neurosci. Res.* **43**: 735-744, 1996.
3. Pandey, A., *et al.*, *J. Biol. Chem.* **270**: 19201-19204, 1995.
4. Pandey, A., *et al.*, *Science* **268**: 567-569, 1995.

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 EDTA; 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 TBS containing 5% nonfat dry milk (Catalog # 20-200) and 0.1% Tween-20 (TBST-MLK) for 20-60 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 μ g/ml of anti-Eck/EphA2, clone D7** diluted in freshly prepared TBST-MLK overnight with agitation at 4 $^{\circ}$ C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:5000 dilution was used) in TBST-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.1% 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).

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