

Anti-Eck/EphA2, clone D7

Monoclonal Antibody

Cat. # 05-480

Lot # DAM1524380

pack size: 200 µg

Store at -20°C

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IP, IC, KA	H, M, R, B, Ca	IgG1	N/A	M	140 kDa	NM_004431

Background

EPH and EPH-related receptors have been implicated in mediating developmental events, particularly in the nervous system. Receptors in the EPH subfamily typically have a single kinase domain and an extracellular region containing a Cys-rich domain and 2 fibronectin type III repeats. The ephrin receptors are divided into 2 groups based on the similarity of their extracellular domain sequences and their affinities for binding ephrin-A and ephrin-B ligands.

Protein kinases are enzymes that transfer a phosphate group from a phosphate donor, generally the γ phosphate of ATP, onto an acceptor amino acid in a substrate protein. By this basic mechanism, protein kinases mediate most of the signal transduction in eukaryotic cells, regulating cellular metabolism, transcription, cell cycle progression, cytoskeletal rearrangement and cell movement, apoptosis, and differentiation.

Presentation

Purified mouse monoclonal IgG1 in buffer containing 0.2 M Tris-glycine, pH 7.4, 0.15 M NaCl, with 0.05% sodium azide and 30% glycerol.

Concentration

1 mg/mL

Specificity

Recognizes Eck/EphA2, Mr 140 kDa

Species Cross-reactivity

Human, mouse, rat, bovine and canine.

Immunogen

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

Molecular Weight

140 kDa

Method of Purification

Protein G Purified

Storage and Handling

Stable for 1 year at -20°C from date of receipt.

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.

Control

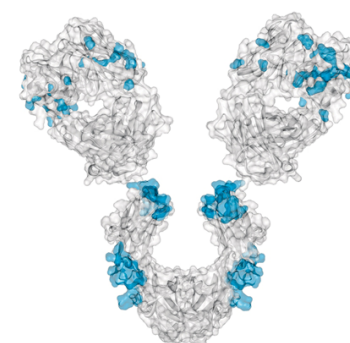
A431 cell lysate, CHO cell lysate, foreskin fibroblasts, 3T3/A31 cells, L6 cells.

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

Quality Control Testing

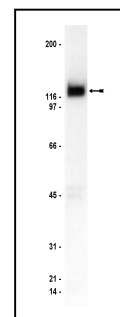
Routinely evaluated by immunoblot on RIPA lysates from human A431, foreskin fibroblasts, murine 3T3/A31 or rat L6 cells.

Western Blot Analysis: 1-2 µg/mL of this lot detected Eck in RIPA lysates from human A431 and previously from foreskin fibroblasts, murine 3T3/A31 and rat L6 cells.



References

- Carles-Kinch, K., et al. (2002). *Cancer Res.* 62:2840-7.
- Hess, A. R., et al. (2001). *Cancer Res.* 61:3250-5.
- Kinch, M. S., et al. (1998). *Hybridoma.* 17:227-35.
- Magal, E., et al. (1996). *J. Neurosci. Res.* 43: 735-744.
- Romer, L., et al. (1994). *Mol. Biol. Cell.* 5: 349-361.



Western Blot Analysis: Representative data from a previous lot.

A431 cell lysate was resolved by electro-phoresis, transferred to nitrocellulose and probed with anti-Eck/EphA2 (0.5 µg/mL). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.

Arrow indicates Eck (~140 kDa).

APPLICATION LEGEND: WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence IH Immunohistochemistry (Tissue) KA Kinase Assay

SPECIES LEGEND: H Human M Mouse R Rat Rb Rabbit B Bovine Ca Canine

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Additional Research Applications

Protein Kinase Assay: A previous lot of this antibody has been reported to have been used in an immunoprecipitation autophosphorylation assay, using a Mn-PIPES reaction buffer (Romer, L., 1994).

Immunoprecipitation: A previous lot of this antibody has been reported to immunoprecipitate Eck from 500 µg 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: A previous lot of 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.

PROTOCOL**Western Blot**

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL each aprotinin, leupeptin, pepstatin; 1 mM Na₃VO₄; 1 mM 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 1-2 µg/mL of anti-Eck/EphA2, clone D7 diluted in freshly prepared TBST-MLK overnight with agitation at 4°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).

RELATED PRODUCTS (specific)

cat #	description
05-543	■ Anti-Eck/EphA2, clone B2D6
12-301	■ Non-Stimulated A431 Cell Lysate
12-349	■ Goat Anti-Mouse IgG, HRP conjugate

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G

■ antibodies ■ Multiplex products ■ biotools ■ cell culture ■ enzymes ■ kits ■ proteins/peptides ■ siRNA/cDNA products

Please visit www.millipore.com for additional product information, test data and references

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