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an Argonex Company

Certificate of Analysis

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Anti-Eck/EphA2, clone B2D6
(mouse ascites)
Catalog # 05-543
Lot # 19377

Immunogen: Native Epithelial Cell Kinase (Eck, also referred to as EphA2 and Mpk-5), isolated by purification of phosphotyrosine-containing proteins from ras-transformed human breast epithelial cells. Clone B2D6.

Specificity: Recognizes Eck/EphA2/Mpk-5, Mr 140kDa.

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

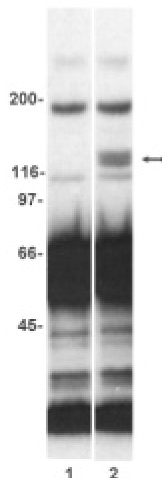
Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

Formulation: 100ml of mouse ascites containing 30% glycerol. Liquid at -20°C.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

Immunoprecipitation: 0.5-2µl of this lot immunoprecipitated EphA2 from 500µg of MCF-7 RIPA lysate. Immunoblot analysis was then performed using 0.5µg/ml of monoclonal Anti-Eck/EphA2, clone D7 antibody (Catalog # 05-480).



Immunoprecipitation/Immunoblot: Anti-Ec/EphA2, clone B2D6 (Catalog # 05-543) was used to immunoprecipitate Eck/EphA2 from MCF-7 cell lysates. The immunoprecipitates were resolved by electrophoresis, transferred to nitrocellulose and probed with no primary antibody (lane 1) or anti-Eck/EphA2, clone D7 (lane 2). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates EphA2 (140 kDa).

Immunoprecipitation/Immunoblot Protocol

1. Pre-clear cell lysate by adding 100µl of protein A agarose, 50% slurry, per ml of lysate. Incubate at 4°C for 10 minutes. Spin and transfer supernatant to a clean tube.
2. Dilute cell lysate using PBS to roughly 1µg/µl total cell protein before beginning the immunoprecipitation, in a microcentrifuge tube.
3. Add **0.5-2ml of anti-Eck/Eph2A, clone B2D6** to 500µg-1mg cell lysate.
4. Gently rock the reaction mixture at 4°C overnight.
5. Add **4mg of anti-Mouse IgG** to 100µl protein A agarose. Gently rock for 30 minutes**.**
6. Capture the immunocomplex by adding the primary antibody mixture to the protein A agarose mixture. Anti-Mouse IgG is used to bridge antibody immunocomplex to protein A agarose.
7. Gently rock the reaction mixture at 4°C for 2 hours.
8. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
9. Resuspend the agarose beads in 60µl 2X Laemmli sample buffer.
10. Collect the beads by a microcentrifuge pulse. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a sample of the supernatant and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
11. Block the blotted nitrocellulose in freshly prepared PBS containing 5% nonfat dry milk, 0.1% Tween 20 (PBST-MLK), for 20 minutes at 20-25°C with constant agitation.
12. Incubate the nitrocellulose with 0.5-1µg/ml of monoclonal anti-Eck/EphA2, clone D7 (Catalog # 05-480), diluted in freshly prepared PBST-MLK overnight with agitation at 4°C.
13. Wash the nitrocellulose with water twice
14. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:3000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
15. Wash the nitrocellulose with water twice.
16. Wash the nitrocellulose in PBS-0.01% Tween 20 for 3-5 minutes.
17. Rinse the nitrocellulose in 4-5 changes of water.
18. Use detection method of choice (enhanced chemiluminescence was used).