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ProductInformation

Monoclonal Anti-RHoGAP (p190)
Clone D2D6
Purified Mouse Immunoglobulin

Product Number **R 3150**

Product Description

Monoclonal Anti-RhoGAP (mouse IgG isotype) was produced from the D2D6 hybridoma produced by the fusion of mouse P3X myeloma cells and splenocytes from a mouse immunized with a GST fusion protein to amino acids 180-610 of rat p190 RhoGAP as immunogen. The antibody is purified from mouse ascites fluid using protein G.

Monoclonal Anti-RhoGAP specifically detects RhoGAP (~190 kDa) by immunoblotting and immunoprecipitation. The antibody cross-reacts with rat, mouse, human, and monkey RhoGAP.

The p190 Rho-GAP protein is associated with p120 RasGAP in growth-factor stimulated and tyrosine kinase transformed cells.[1] It functions as a GTPase – activating protein (GAP) for Rho and Rac family proteins, which are involved in regulating cytoskeletal actin and membrane ruffling.[2] The antibody appears to couple signal transduction via Ras and Rho through its association with RasGAP.[3] The presence of two adjacent SH2 domains in the p21ras GAP indicates that GAP might interact directly with tyrosine kinases.[4]

Reagent

The antibody is supplied as approx. 200 µg of purified mouse immunoglobulin in 0.1 M Tris-glycine, pH 7.4, containing 0.15 M sodium chloride, and 0.05% sodium azide.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

Store at –20 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedures

Immunoprecipitation

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1 µg/µl total cell protein in a microcentrifuge tube with PBS (Product No. P 3813).
2. Add 4.0µg of Anti-RhoGAP(p190) to 500 µg-1 mg cell lysate.
3. Gently rock the reaction mixture at 4 °C overnight.
4. Capture the immunocomplex by adding 100 µl of washed (in PBS) 1:1 slurry of Protein G-Agarose beads (50µl packed beads) (Product No. P 2294).
5. Gently rock reaction mixture at 4°C for 2 hours.
6. 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 (see below) or PBS.
7. Resuspend the agarose beads in 60µl 2X Laemmli sample buffer.
8. The agarose beads can be frozen for later use or suspended in Laemmli sample buffer and boiled for 5 minutes. Pellet the beads using a microcentrifuge pulse. SDS-PAGE and subsequent immunoblotting analysis may be performed on a sample of the supernatant.

Lysis Buffer:
50 mM Tris-HCl, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 µg/ml each aprotinin, leupeptin, pepstatin, 1 mM Na_3VO_4, and 1 mM NaF.

Product Profile
Recommended working concentration for immunoblotting is 0.5-2 µg/ml of Monoclonal Anti-RhoGAP using RIPA lysates from human A431 carcinoma cells, mouse 3T3 fibroblasts, and rabbit brain membrane extract, anti-Mouse IgG conjugated to Peroxidase and enhanced chemiluminescence.

For immunoprecipitation, 4 µg of this antibody has been used to immunoprecipitate RhoGAP from 500 µg of human A431 carcinoma cell RIPA lysates.

Note: In order to obtain the best results and assay sensitivity in various techniques and preparations, we recommend determining optimal working dilutions by titration test.

References

1. Settleman, J., et al., Nature, **359**, 153 (1992).
2. Cheng, J.C., et al., Cell Growth Differ., 6, 139 (1995).
3. Foster, R., et al., Mol. Cell Biol., **14**,: 7173 (1994).
4. Ellis, C., et al., Nature, **343**,: 377 (1990).

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