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Product Information

Anti-Rho-GDI
produced in rabbit, IgG fraction of antiserum

Catalog Number **R3025**

Product Description

Anti-Rho-GDI is produced in rabbit using a GST fusion protein corresponding to full length human Rho-GDI as immunogen. Anti-Rho-GDI is purified using protein A.

Anti-Rho-GDI recognizes Rho-GDI and cross-reacts with D4-GDI. The antibody reacts with human, bovine and mouse Rho-GDI. The antibody will identify Rho-GDI by immunoblotting (28 kDa) and immunoprecipitation.

GDP- Dissociation inhibitors (GDIs) modulate GTPase activity by binding to small G-proteins and regulating GDP/GTP exchange of inflammatory cell activity, including motility, formation of toxic oxygen metabolites and generation of proinflammatory cytokines.[1] Rho-GDI may also be critical for the cellular compartmentalization of GTPases.[2] Rho-GDI, which can bind to Rho, Rac, and cdc42, is localized in the cystol.[3] D4-GDI, an abundant hematopoietic cell GDI for the Ras-related Rho family GTPases, is a substrate of the apoptosis protease.[4]

Reagent

Supplied as a solution in 0.1 M Tris-glycine, pH 7.4, containing 0.15 M NaCl and 0.05% sodium azide.

Protein concentration: ~1 mg/ml.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Procedures

Immunoprecipitation

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1 µg/ml total cell protein in a microcentrifuge tube with PBS, Catalog Number P3813.
2. Add 4 µg of Anti-Rho-GDI to 0.5-1 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 A-Agarose beads. 50 µL packed beads; Catalog Number P2545.
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 50µL 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

The recommended working concentration is 0.5-1.0 µg/ml for immunoblotting using RIPA lysates from human HL-60, HeLa, Hs294T, Jurkat and murine 3T3/NIH cells and mouse 3T3/A31. Visualized by an anti-rabbit IgG-peroxidase and a chemiluminescent detection system.

For immunoprecipitation, 4 µg is recommended to immunoprecipitate Rho-GDI from 500 µg of Jurkat RIPA lysate.

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

References

1. Danley, D.E., et al., *J. Immunol*., **157**, 500 (1996).
2. Adra, C. N., et al., *Proc. Natl. Acad. Sci. USA*, **94**, 4279 (1997).
3. Zalcman, G., et al., *J. Biol. Chem*., **271**, 30366 (1996).
4. Na, S., et al., *J. Biol. Chem*., **271**, 11209 (1996).

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