

Product Information

ANTI-D4-GDI

Developed in Rabbit,
IgG fraction of Antiserum

Product Number **D9937**

Product Description

Anti-D4-GDI is developed in rabbit using synthetic peptide (KAPEPHVEEDDDDELDSKC) corresponding to amino acids 4-21 of human D4-GDI conjugated to KLH as immunogen. The IgG fraction of antiserum is purified using protein A.

Anti-D4-GDI specifically reacts with D4-GDI and does not recognize Rho-GDI or GDI-3. It reacts with human and bovine D4-GDI. The antibody will identify D4-GDI by immunoblotting (28 kDa) and immunoprecipitation.

GDP-dissociation inhibitors or GDIs, modulate the activation of GTPases, and may also be involved in the cellular compartmentalization of GTPases. GDIs modulate GTPase activity by binding to small G Proteins, and regulating GDP/GTP exchange. This family of proteins includes Rho-GDI (GDI-1), D4-GDI (GDI-2) and Rho-GDI-3 (GDI-3). Rho-GDI and D4-GDI are both localized in cytosol. RhoGDI and GDI/D4 are both members of the Rho-subfamily of GTPases. These two proteins are highly homologous, differing primarily in the first 25 amino acids of the N-terminus. D4-GDI has been described as a Caspase 3 (CPP32/YAMA/Apopain) substrate during Fas-induced apoptosis and is located in the cytosol.

Reagents

The product is supplied as the IgG fraction of antiserum in 0.07M Tris-glycine, pH 7.4, containing 0.105 M NaCl, 30% glycerol and 0.035% sodium azide. Antibody concentration is approximately 0.7 mg/ml.

Precautions and Disclaimer

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

Storage/Stability

Store at 0°C to +20°C. 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 (Sigma Product No. P3813).
2. Add 4 µg of Anti-D4-GDI (D 9937) 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) (Sigma Product No. 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 50 µl 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 Na₃VO₄, and 1 mM NaF.

Product Profile

Recommended working concentration is 0.5-1 µg/ml for immunoblotting using RIPA lysates from human A431, HL-60 and Jurkat cells, anti-rabbit IgG-peroxidase conjugate and a chemiluminescent detection system.

For IP, 4 µg is recommended to immunoprecipitate D4-GDI from 500 µg of a 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. Adra, C., et al., Proc. Natl. Acad. Sci. USA, **94**, 4279 (1997).
2. Zalcman, G., et al., J. Biol. Chem., **271**, 30366 (1996).
3. Adra, C.N., et al., Genomics, **53**, 104 (1998).
4. Danley, D.E. et al., J. Immunol., **271**, 500, (1996).
5. Na, S. et al., J. Biol. Chem., **271**, 11209, (1996).

jo/mms/lpg 2/00

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.