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ProductInformation

ANTI-GI ALPHA 3 ($G_{i\alpha 3}$)
Developed in Rabbit,
Whole Antiserum

Product Number **G 4040**

Product Description

Anti-Gi alpha 3 is developed in rabbit using a 10 residue, synthetic peptide corresponding to the C-terminal region of Gi alpha 3 (KNNLKECGLY) as immunogen.

Anti-Gi alpha 3 recognizes Gi alpha 3 (approximately 40 kDa) and Go alpha G-proteins by immunoblotting and immunoprecipitation. In addition this antibody has low cross-reactivity with other G-proteins. The antibody reacts with human, rat, mouse, and hamster Gi alpha 3 and Go alpha. This antibody can be used for the detection in both immunoblotting as well as immunoprecipitation.

Gi alpha 3 is a G-protein subunit that is involved in many signal transduction pathways including the mediation of EGF-induced PLC-γ activation and Ca^{+2} mobilization in hepatocytes[1,2]. G-proteins are membrane associated heterotrimeric proteins that are comprised of α-, β-, and λ-subunits. The α-subunit contains a guanine-binding domain that is in its inactive state when it is occupied by GDP. Upon activation, GDP is replaced with GTP, causing the dissociation of the α-subunit from the βλ-subunit complex. This enables the Gα-GTP complex to bind to and regulate specific signaling pathways. GTP is then hydrolyzed, allowing for re-association of the α-subunit with the βλ-subunit complex.

Reagents

Anti-Gi alpha 3 is supplied as whole antiserum, Each vial contains approximately 50 µl.

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.

Procedure

Immunoprecipitation

1. Add 500 mg-1 mg of a solubilized membrane lysate, at a concentration of roughly 1mg/ml total cell protein, to a microcentrifuge tube.
2. Add 4µl of Anti-Gi alpha 3 (G 4040) to the tube.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 µl of washed Protein A/G agarose bead slurry (50 µl packed beads),
5. Gently rock the 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 ice-cold cell lysis buffer (see below) or PBS.
7. Resuspend the agarose beads in 50 µl 2x Laemmli sample buffer.
8. The agrose beads can either be frozen for later use or suspended in Laemmli sample buffer and boiled for 5 minutes. Collected the beads by microcentrifuge pulse and SDS-PAGE. Subsequent Immunoblot analysis can 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 dilution is 1:2,000 for immunoblotting using solubilized mouse and rat brain membrane lysates, and mouse 3T3 cell lysates, anti-rabbit IgG-peroxidase conjugate and a chemiluminescent detection system.

For IP, 4 µl is recommended to precipitate type II transglutaminase from solubilized mouse brain membrane lysates.

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. 1.Yang, L.J., et al., J. Biol. Chem., **266**, 22451 (1991).
2. Lynch, C.J., et al., J. Clin. Invest., **83**, 2050 (1989).

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