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ProductInformation

MONOCLONAL ANTI-GRK 4-6
CLONE A16/17
Purified Mouse Immunoglobulin

Product Number **G4540**

Product Description

Monoclonal Anti-GRK 4-6 (mouse IgG1κ isotype) was produced using a GST fusion protein corresponding to residues 463-590 of bovine GRK5 as immunogen. This sequence shares 95% homology with human and rat GRK5 and approximately 80% homology with human and rat GRK4 and GRK6. The antibody is purified using ammonium sulfate precipitation.

Monoclonal Anti-GRK 4-6 specifically reacts with human, mouse and rat GRK 4-6. It may be used to identify GRK4, GRK5 and GRK6 by immunoblotting[1] and immunoprecipitation.

G-protein coupled receptor kinases (GRKs) are a family of serine/threonine kinases that play a role in desensitization by phosphorylation of G protein-coupled receptors. Receptors are phosphorylated by GRKs in vitro on intracellular loops and/or the carboxyl-terminal tail. The GRK-phosphorylated receptors are bound by arrestin proteins, leading to the functional removal of the receptor from the signaling pathway by preventing further receptor coupling to G proteins.[2]
There are three groups of G protein coupled receptors: GRK1 (rhodopsin kinase), GRK2-like (GRK2/3), and GRK4-like (GRK4/5/6). The GRK-2 like and GRK-4 like proteins have the highest sequence similarity only within the central protein kinase catalytic domain. There is however, considerable sequence divergence among the six GRKs in the regulatory amino- and carboxyl-terminal domains, which may account for the differences in regulation among the three kinase subfamilies. Thus GRK2-like kinases are translocated to the plasma membrane following receptor activation by interaction of the carboxyl-terminal domain with G protein -subunits and membrane phosphatidyl inositol 4,5-bisphosphate,[3,4] while GRK4-like kinases exhibit high constitutive membrane association.[5,6] Although both GRK2-like and GRK4-like kinases are regulated by membrane lipids, the site and mechanism of action differ between the two groups.[7] GRKs are regulated by additional mechanisms such as the calcium-dependent regulation. Recent studies have demonstrated that GRK2 can be phosphorylated by PKC, resulting in an ~2-3-fold activation of the kinase, possibly via an increased association of GRK2 with membranes.[8] Conversely, GRK5 is significantly inhibited after phosphorylation by PKC because of a decreased activity and decreased affinity for receptor.[9] In the visual system, GRK1 has been shown to be inhibited by the Ca2+-binding protein recoverin.[10] Although regulation by recoverin may be specific for GRK1, a universal mediator of calcium signaling, calmodulin, appears to inhibit all other GRK subtypes, with GRK5 being the most sensitive.

Reagents

The product is supplied as purified mouse immunoglobulin in 0.1M Tris-glycine, pH 7.0, containing 0.035% sodium azide and 30% glycerol
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 0EC to !20EC. 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 1µg/ml total cell protein in a microcentrifuge tube with PBS (Sigma Product No. P3813).
2. Add 4 µg of Monocloanl Anti-GRK 4-6 (G 4540) to 0.5 - 1 mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 2 µg rabbit anti-mouse IgG bridge antibody and 100 µl of washed protein A agarose bead slurry (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 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 1-2 µg/ml for immunoblotting using RIPA lysates from human K562 cells and murine 3T3 cells, anti-mouse IgG-peroxidase conjugate and a chemiluminescent detection system.

Note: This antibody does not blot when diluted in a PBS based dilution buffer. Use TBS containing 5% milk and 0.05% Tween-20.

For IP, 4 µg is recommended to immunoprecipitate GRK4-6 from 500 µg of a human K562 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

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