

Product Information

MONOCLONAL ANTI-RASGAP Clone B4F8 Purified Mouse Immunoglobulin

Product Number **R2775**

Product Description

Monoclonal Anti-Ras GTPase-activating protein (RasGAP) (mouse IgG isotype) is derived from the B4F8 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/3T3 immunized with full-length recombinant human GAP expressed in *Sf9* insect cells. The antibody is produced in cell culture and purified using Protein G.

Monoclonal Anti-RasGAP specifically reacts with human GAP by immunoblotting (120 kDa), immunocytochemistry and immunoprecipitation. It cross-reacts with rat, mouse, bovine, and hamster GAP.

The Ras-related low molecular mass GTPases participate in signal transduction involving a variety of cellular functions, including cell-cycle progression, cellular differentiation, cytoskeletal organization, protein transport and secretion.¹ The Ras-GTPase-activating protein (RasGAP) forms a complex with a second protein, p190, in growth factor stimulated and tyrosine-kinase transformed cells.¹ The RasGAP p190 complex may serve to couple Ras- and Rho-mediated signaling pathways.² RasGAP forms an abundant SH2-mediated complex with p190 RhoGAP in cells expressing activated tyrosine kinases.³

Reagents

The product is supplied as purified mouse immunoglobulin 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 mg/ml total cell protein in a microcentrifuge tube with PBS (Product No. P3813).
2. Add 1-5 µg of Monoclonal Anti-RasGAP (R2775) to 500 µg to 1 mg cell lysate.
3. Gently rock the reaction mixture at 4 °C overnight.
4. Capture the immunocomplex by adding 5 µg of rabbit α-mouse IgG antibody and 100 µl of washed (in PBS) 1:1 slurry of Protein A-Agarose beads (50 µl packed beads) (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 or PBS.
7. Resuspend the agarose beads in 50 µl of 2× 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.

Immunocytochemistry

1. Plate approximately 200 µl of a cell suspension into each well of a slide. Incubate 24 hours in a 37 °C CO₂ incubator.
2. Wash the cells 3 times for 5 minutes with PBS. Do not shake.
3. Add fixative (ice-cold ethanol/acetic acid, (95:5) for 1 minute at room temperature.

4. Wash the cells with PBS, 2 times for 15 minutes with gentle agitation. Do not shake.
5. Add 400 μ l of PBS containing 8% BSA and incubate 30 minutes at room temperature.
6. Wash cells with PBS for 15 minutes.
7. Incubate the cells with 10 μ g/ml of anti-RasGAP in PBS containing 1% BSA and incubate overnight at 4 °C.
8. Wash the cells 2 times with PBS for 5 minutes.
9. Incubate the cells with a 1:100 dilution of goat anti-mouse IgG conjugated with FITC (Product No. F5262) in PBS for 1.0 hour at room temperature.
10. Wash the cells 3 times with PBS for 5 minutes.
11. Examine the cells under a fluorescent microscope.

Product Profile

The recommended working concentration is 1 μ g/ml for immunoblotting using 10 μ g of an A431 cell lysate, anti-mouse IgG-peroxidase conjugate and a chemiluminescent detection system.

For IP, 1 μ g is recommended to precipitate GAP from 250 μ g of an A431 cell lysate.

A working concentration of 10 μ g/ml is recommended to detect GAP in ethanol:acetic acid-fixed [50:50] A431 cells.

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. Settleman, J., et al., *Nature*, **359**, 153-154, 1992.
2. Foster, R., et al., *Mol. Cell Biol.*, **14**, 7173-7181, 1994.
3. Hu, KQ and Settleman, J., *EMBO J.*, **16**, 473-483, 1997.

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