

Product Information

Monoclonal Anti-Rac, Clone 23A8

produced in mouse, purified immunoglobulin

Catalog Number **R2650**

Product Description

Monoclonal Anti-Rac (mouse IgG2b isotype) was produced using as immunogen a recombinant protein containing the full length human Rac1. The antibody was purified using Protein G.

Monoclonal Anti-Rac recognizes Rac1 with a slight Rac2 cross-reactivity. It recognizes human, mouse, and rat Rac. Anti-Rac may be used in various techniques such as immunoblotting (21 kDa), immunoprecipitation, and immunohistochemistry.

Rac, a small GTPase in the ras family, regulates the formation of membrane ruffles, lamellipodia and filopodia.^{1,2} It is involved in cytoskeletal actin organization and transformation. The p21 activated kinases (PAKs) are direct targets of active Rac and Cdc42, which can induce the assembly of polarized cytoskeletal structures when expressed in fibroblasts.² Rac1 and Rac2 are also essential components of NADPH oxidase, the enzyme responsible for generating free radicals.³

Reagent

Supplied in 0.1 M Tris-glycine, pH 7.4, containing 0.15 M NaCl, 0.05% sodium azide and 30% glycerol.

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

Store at -20 °C. 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 ~1 mg/ml total cell protein in a microcentrifuge tube with Phosphate buffered saline, Catalog Number P3813.
2. Add 4 µg of Monoclonal Anti-Rac to the tube containing the 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 G-Agarose beads (50 µL packed beads), Catalog Number P7700.
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 60 µL 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.

Cell Lysis Buffer:

50 mM Tris-HCl, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1.0 mM PMSF, 1 µg/ml each aprotinin, leupeptin, pepstatin, 1 mM Na₃VO₄, and 1 mM NaF.

Immunohistochemistry

1. Cover cryostat sections with fixative, ice cold 4% paraformaldehyde/2% acetic acid in PBS, and incubate for 30 minutes at room temperature.
2. Wash sections with PBS for 5 minutes with gentle agitation.
3. Cover the sections with the secondary fixative, ice cold ethanol: acetic acid (95:5), for 2 minutes at room temperature.
4. Wash the sections with PBS, twice for 15 minutes. Do not shake.
5. Cover the sections with 8% BSA in PBS and incubate 30 minutes at room temperature.
6. Wash cells with PBS for 15 min.
7. Incubate the cells with 10 µg/ml of Monoclonal Anti-Rac in PBS containing 1% BSA and incubate for 1 hour at room temperature or at 4 °C overnight.
8. Wash the cells 2× with PBS for 30 min.
9. Incubate the cells with a 1:100 dilution of Anti-Mouse IgG (Fab specific)-FITC, Catalog Number F5262, in PBS for 3 hours at room temperature.
10. Wash the cells 3× with PBS for 30 min.
11. Examine the cells under a fluorescent microscope.

Product Profile

Immunoblotting: the recommended working concentration is 0.5-2 µg/mL using rat brain microsomal protein preparation, Anti-Mouse IgG-Peroxidase and a chemiluminescent detection system.

Immunoprecipitation: 4 µg is recommended to precipitate Rac from a rat brain microsomal protein preparation.

Immunohistochemistry: a working concentration of 10 µg/ml is recommended to detect Rac in rat brain fixed with 4% paraformaldehyde, 2% acetic acid in phosphate buffered saline for 30 minutes followed by ethanol:acetic acid (95:5) for 2 minutes.

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

References

1. Diekmann, D., et al., *EMBO J*, **14**, 5297-5305, 1995
2. Dharmawardhane, S., et al., *J Cell Biol.*, **138**, 1265-1278, 1997
3. Razzouk, S., et al., *Eur Cell Biol.*, **78**, 249-255,

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