

ORDERING INFORMATION

Catalog Number: MAB3270

Clone: 399527

Lot Number: YXP01

Size: 100 µg (sufficient for 100 mL of blotting solution)

Formulation: 0.2 µm filtered solution in PBS with 5% trehalose

Storage: -20° C

Specificity: human/mouse/rat RalA

Immunogen: *E. coli*-derived rhRalA

Ig class: rat IgG_{2a}

Recommended Application:
Western blot

Background

The Ras-like proteins RalA and RalB share 85% identity and constitute a grouping within the Ras superfamily of small GTPases. Like other GTPases, Ral proteins transduce signals by cycling between an active GTP-bound and an inactive GDP-bound state. Ral functions as an effector of Ras-mediated signaling, and has been implicated in the regulation of vesicle trafficking and cell morphology.

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with purified, *E. coli*-derived full-length recombinant human RalA (rhRalA; aa 1 - 206; accession # P11233). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute with sterile PBS. If 0.2 mL of PBS is used, the antibody concentration will be 500 µg/mL.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a **manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody detects endogenous human, mouse, and rat RalA at 25 kDa using Western blot. The antibody does not cross-react with recombinant human RalB.

Application

Western Blot - An antibody concentration of 1.0 µg/mL is recommended.

Optimal dilutions should be determined by each laboratory for each application.

Protocols for Immunoblotting

Blotting Buffer

25 mM Tris, pH 7.4
0.15 M NaCl
0.1% Tween® 20

Blocking Solution

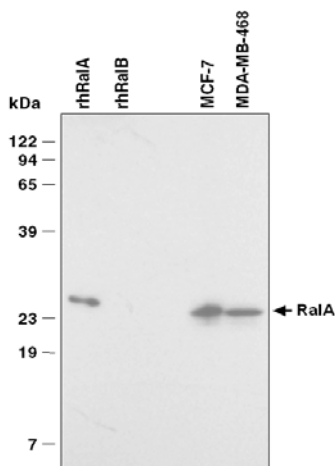
2% nonfat dry milk in
Blotting Buffer
Adjust pH to 7.4

Antibody Solution

2% nonfat dry milk in
Blotting Buffer
Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 1.0 µg/mL rat anti-human/mouse/rat RalA.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1000 dilution of HRP-conjugated goat anti-rat IgG (Zymed).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with WesternGlo™ Chemiluminescent detection reagents (R&D Systems, Catalog # AR004) or equivalent.

Cell lysates for Western blottings: To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at 2 x 10⁶ - 1 x 10⁷ cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each.



Detection of RalA with MAB3270.

Two ng of rhRalA and rhRalB, and lysates of human MCF-7 and MDA-MB-468 cells were resolved by SDS-PAGE. Following electrophoresis, proteins were transferred to an Immobilon-P membrane and immunoblotted with 1.0 µg/mL anti-RalA, as described in *Protocols for Immunoblotting*. A 25 second exposure to film is shown.