

# Affinity-Purified Goat Anti-human/mouse/rat RalA/B Antibody

## ORDERING INFORMATION

**Catalog Number:** AF3204

**Lot Number:** WIS01

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

**Storage:** -20° C

**Specificity:** human/mouse/rat RalA/B

**Immunogen:** *E. coli*-derived rhRalB

**Ig Type:** goat IgG

**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

Goat antibodies were raised against purified, *E. coli*-derived full-length recombinant human RalB (rhRalB; aa 1 - 206; accession # P11234). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified by isolating the IgG fraction.

## Formulation

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

## Reconstitution

Reconstitute in PBS containing 0.02% NaN<sub>3</sub>.

## 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 and RalB at 25 kDa using Western blot. The antibody does not cross-react with recombinant human H-Ras, N-Ras, or K-Ras.

## Application

For Western blot analysis, an antibody concentration of 1.0 µg/mL is recommended.

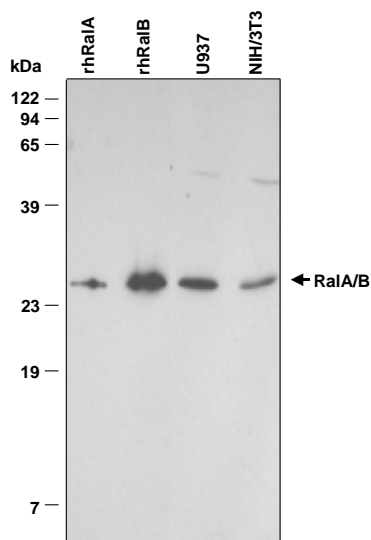
**Optimal dilutions should be determined by the individual laboratory.**

## Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk in Blotting Buffer	5% nonfat dry milk in Blotting Buffer
0.15 M NaCl	Adjust pH to 7.4	Adjust pH to 7.4
0.1% Tween 20		

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 anti-human/mouse/rat RalA/B.
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 1:1,000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with WesternGlo Chemiluminescent Detection Reagent (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 10<sup>6</sup> - 1 x 10<sup>7</sup> 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 and RalB with AF3204.** 5 ng of rhRalA and rhRalB, and lysates from human U937 and mouse NIH/3T3 cells were resolved by SDS-PAGE, transferred to an Immobilon-P membrane, and immunoblotted with 1.0 µg/mL anti-RalA/B, as described in *Protocols for Immunoblotting*. A one minute exposure to film is shown.