

ORDERING INFORMATION

Catalog Number: AF3434

Lot Number: WUB01

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

Storage: -20° C

Specificity: human/mouse/rat RACK1

Immunogen: *E. coli*-derived rhRACK1

Ig Type: goat IgG

Applications: Western blot
Immunocytochemistry

Background

The receptor for activated C kinase 1 (RACK1) is highly conserved and has homology to the β subunit of heterotrimeric G proteins. Initially isolated by its binding of active PKC isoforms, RACK1 is a scaffold protein that promotes the formation of multiprotein complexes that integrate distinct signaling pathways. RACK1 is also a 40S ribosomal subunit constituent, potentially linking these pathways directly to protein translation.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived full-length recombinant human RACK1 (rhRACK1; aa 1 - 317; Accession # P63244). 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% NaN₃.

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 RACK1 at 36 kDa using Western blot.

Applications

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

Immunocytochemistry - This antibody will detect RACK1 in cells. The working dilution is 2 - 15 µg/mL. For chromogenic detection of labeling, use R&D Systems' Cell and Tissue Staining Kits (CTS Series).

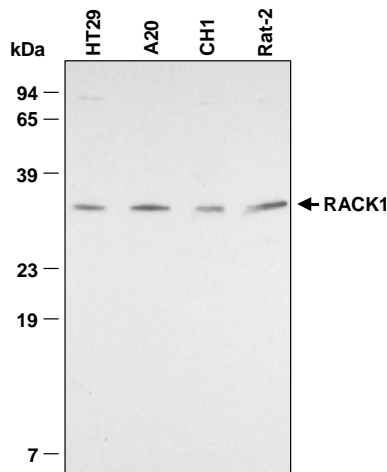
Optimal dilutions should be determined by each laboratory for each application.

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 RACK1.
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 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 RACK1 with AF3434.

Lysates from human HT29, mouse A20, mouse CH1, and rat Rat-2 cells were resolved by SDS-PAGE, transferred to an Immobilon-P membrane, and immunoblotted with 1.0 µg/mL anti-RACK1, as described in *Protocols for Immunoblotting*. A twenty second exposure to film is shown.