

#2098 Store at -20°C

RhoB Antibody

✓ 100 µl
(10 Western mini-blot)

Orders ■ 877-616-CELL (2355)
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New 04/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.

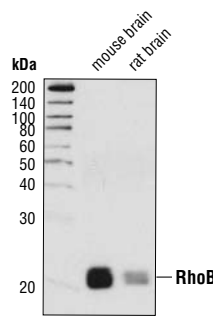
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, M, R, Mk	21 kDa	Rabbit

Background: Rho family small GTPases, including Rho, Rac and cdc42, act as molecular switches, regulating processes such as cell migration, adhesion, proliferation and differentiation. They are activated by guanine nucleotide exchange factors (GEFs), which catalyze the exchange of bound GDP for GTP, and inhibited by GTPase activating proteins (GAPs), which catalyze the hydrolysis of GTP to GDP. A third level of regulation is provided by the stoichiometric binding of Rho GDP dissociation inhibitor (RhoGDI) (1). RhoA, RhoB and RhoC are highly homologous, but appear to have divergent biological functions. Carboxy-terminal modifications and differences in subcellular localization allow these three proteins to respond to and act on distinct signaling molecules (2,3).

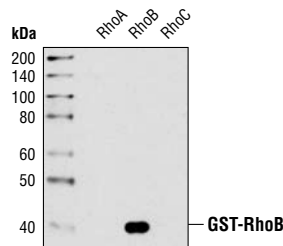
RhoB functions in the regulation of cell shape, migration and adhesion (4). RhoB activity has also been shown to play a role in protein trafficking (5,6) and in CXCR2-mediated chemotaxis (6). Inhibition of RhoB activity downstream of PKC ζ influences the degree of invasion and migration by glioblastoma cells (7), and RhoB expression has a negative effect on tumor growth in ovarian cancer (8).

Specificity/Sensitivity: RhoB Antibody detects endogenous levels of total RhoB protein. The antibody does not cross-react with RhoA or RhoC.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues near the carboxy terminus of human RhoB. Antibodies are purified using protein A and peptide affinity chromatography.



Western blot analysis of extracts from mouse and rat brain tissue using RhoB Antibody.



Western blot analysis of recombinant GST-tagged RhoA, RhoB and RhoC using RhoB Antibody.

Entrez-Gene ID #388
Swiss-Prot Acc. #P62745

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by Western blot.

Recommended Antibody Dilutions:

Western blotting 1:1000

Companion Products:

- RhoA (67B9) Rabbit mAb #2117
- Cdc42 Antibody #2462
- Rac1/2/3 (L129) Antibody #2467
- Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 20X LumiGLO® Reagent and 20X Peroxide #7003

Background References:

- (1) DerMardirossian, C. and Bokoch, G.M. (2005) *Trends Cell Biol* 15, 356–63.
- (2) Wennerberg, K. and Der, C.J. (2004) *J Cell Sci* 117, 1301–12.
- (3) Wheeler, A.P. and Ridley, A.J. (2004) *Exp Cell Res* 301, 43–9.
- (4) Wheeler, A.P. and Ridley, A.J. (2007) *Exp Cell Res* 313, 3505–16.
- (5) Sandilands, E. et al. (2007) *J Cell Sci* 120, 2555–64.
- (6) Neel, N.F. et al. (2007) *J Cell Sci* 120, 1559–71.
- (7) Baldwin, R.M. et al. (2008) *Oncogene*, Epub ahead of print.
- (8) Couderc, B. et al. (2008) *Cancer Gene Ther*, Epub ahead of print.

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E—ELISA D—Delfia®
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.