Store at -20°C

#9968

Rho-GTPase Antibody Sampler Kit

☑ 1 Kit
(6 x 20 µl)

Cell Signaling TECHNOLOGY®

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rev. 06/16

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Cdc42 (11A11) Rabbit mAb	2466	20 µl	21 kDa	Rabbit IgG
Phospho-Rac1/cdc42 (Ser71) Antibody	2461	20 µl	28 kDa	Rabbit IgG
Rac1/2/3 Antibody	2465	20 µl	21 kDa	Rabbit IgG
RhoA (67B9) Rabbit mAb	2117	20 µl	21 kDa	Rabbit IgG
RhoB Antibody	2098	20 µl	21 kDa	Rabbit IgG
RhoC (D40E4) Rabbit mAb	3430	20 µl	21 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

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 antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000
See www.cellsignal.com for individual component dilutions and additional application protocols.

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Description: The Rho-GTPase Antibody Sampler Kit contains reagents to examine aspects of cell migration, adhesion, proliferation and differentiation in cells. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: The Rho family of small GTPases, including Rho, Rac and cdc42, act as molecular switches to regulate processes such as cell migration, adhesion, proliferation and differentiation (1). RhoA, RhoB and RhoC are all highly homologous but appear to have divergent biological functions. The best characterized of these proteins, RhoA, regulates actomysin contractility, cytokinesis, focal adhesion assembly and cell polarity (2–5). Mammalian Rac exists as three isoforms (Rac1, Rac2 and Rac3) that show high sequence similarity. Rac1 and cdc42 are ubiquitously expressed and play key signaling roles in cytoskeletal reorganization, membrane trafficking, transcriptional regulation and cell growth and development (6). Phosphorylation of Rac1 at a putative Akt site (Ser71) may limit Rac1 activity through inhibition of GTP binding (7). Rac2 is expressed in cells of hematopoietic origin, while Rac3 is highly expressed in brain and in many other tissues. The Vav family of guanine-nucleotide exchange factors mediates activation of Rho/Rac family small GTPases (8). Negative regulation of Rho-activity members of the p190 RhoGAP family (p190-A and p190-B) may be controlled by Src phosphorylation of Tyr residues, activating the p190 GAP domain (8–10). Furthermore, Rho GDP dissociation inhibitor (RhoGDI) associates with Rho/Rac to negatively regulate nucleotide exchange membrane localization (11).

Specificity/Sensitivity:
Cdc42 (11A11) Rabbit mAb detects endogenous levels of total Cdc42 protein and does not cross-react with other small GTPases.
Phospho-Rac1/cdc42 (Ser71) Antibody detects endogenous Rac1/cdc42 only when phosphorylated at Ser71 and may also recognize phospho-RhoA (Ser73).
Rac 1/2/3 (L129) Antibody detects endogenous levels of total Rac1/2/3 proteins.
RhoA (67B9) Rabbit mAb recognizes endogenous levels of total RhoA protein.
RhoB Antibody recongnizes endogenous levels of RhoB.
RhoC (D40E4) XP™ Rabbit mAb recongnizes endogenous levels of RhoC.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Thr138 of human Rac1, corresponding to residues surrounding Ser71 of human Rac1/cdc42, and corresponding to residues near the carboxy terminus of RhoB . Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Monoclonal antibody is produced by immunizing animals with synthetic peptides corresponding to residues surrounding Lys135 of human Cdc42, residues near the carboxy terminus of human RhoA, and to residues corresponding to the carboxy terminus of human RhoC

Background References:

(1) DerMardirossian, C. and Bokoch, G.M. (2005) *Trends Cell Biol* 15, 356–363.

(2) Bi, D. et al. (2005) *Circ. Res.* 96, 890-897.

(3) Kimura, K. et al. (2000) *J. Biol. Chem.* 275, 17233–17236.

(4) Barry, S.T. and Critchley, D.R. (1994) *J. Cell Sci.* 107 (Pt 7), 2033–2045.

(5) Van Keymeulen, A. et al. (2006) *J. Cell Biol.* 174, 437–445.

(6) Wennerberg, K. and Der, C.J. (2004) *J. Cell Sci.* 117, 1301–1312.

(7) Kwon, T. et al. (2000) *J. Biol. Chem.* 275, 423–428.

(8) Sordella, R. et al. (2003) *Cell* 113, 147–158.

(9) Chang, J.H. et al. (1995) *J. Cell Biol.* 130, 355–368.

(10) Roof, R.W. et al. (1998) *Mol. Cell Biol.* 18, 7052–7063.

(11) Dovas, A. and Couchman, J.R. (2005) *Biochem J.* 390, 1–9.

U.S. Patent No. 5,675,063

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.