

# Rho-GTPase Antibody Sampler Kit

✓ 1 Kit  
(6 x 40 µl)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.  
This product is not intended for use as a therapeutic or in diagnostic procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Cdc42 (11A11) Rabbit mAb	2466	40 µl	21 kDa	Rabbit IgG
Phospho-Rac1/cdc42 (Ser71) Antibody	2461	40 µl	28 kDa	Rabbit IgG
Rac1/2/3 (L129) Antibody	2467	40 µl	21 kDa	Rabbit IgG
RhoA (67B9) Rabbit mAb	2117	40 µl	21 kDa	Rabbit IgG
RhoB Antibody	2098	40 µl	21 kDa	Rabbit IgG
RhoC (D40E4) XP™ Rabbit mAb	3430	40 µl	21 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** The Rho-GTPase Antibody Sampler Kit contains reagents to examine aspects of cell migration, adhesion, proliferation and differentiation in cells. This kit includes enough primary and secondary antibodies to perform four Western mini-blot experiments per 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 actomyosin 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** recognizes endogenous levels of RhoB.

**RhoC (D40E4) XP™ Rabbit mAb** recognizes endogenous levels of RhoC.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to the sequence of human Rac1/2/3, 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 antibodies are produced by immunizing rabbits with synthetic peptides (KLH-coupled) 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.

**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:

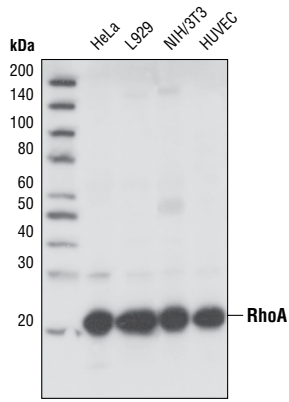
Western blotting 1:1000

See [www.cellsignal.com](http://www.cellsignal.com) for individual component dilutions and additional application protocols.

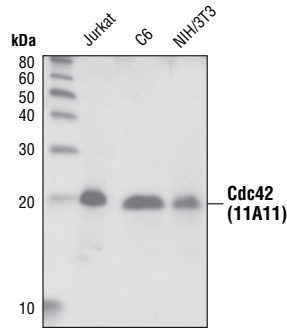
Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

#### Background References:

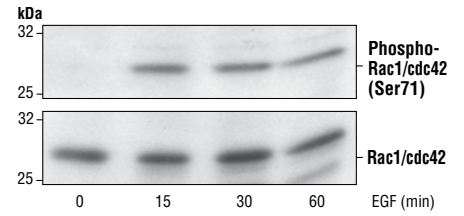
- (1) DerMardirossian, C. and Bokoch, G.M. (2005) *Trends Cell Biol* 15, 356–363.
- (2) Bi, D. et al. (2005) *Circ. Res.* 96, 890–897.
- (3) Kimura, K. et al. (2000) *J. Biol. Chem.* 275, 17233–17236.
- (4) Barry, S.T. and Critchley, D.R. (1994) *J. Cell Sci.* 107 (Pt 7), 2033–2045.
- (5) Van Keymeulen, 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.



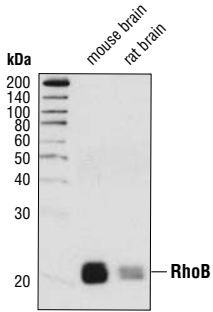
Western blot analysis of extracts from various cell types using **RhoA (67B9) Rabbit mAb #2117**.



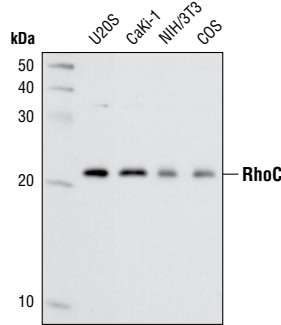
Western blot analysis of extracts from various cell types using **Cdc42 (11A11) Rabbit mAb #2466**.



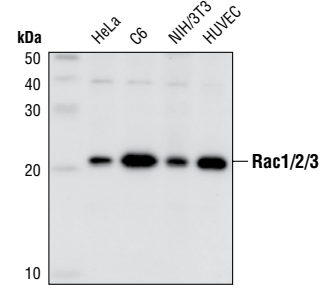
Western blot analysis of extracts from A431 cells treated with EGF for the indicated times using **Phospho-Rac1/cdc42 (Ser71) Antibody #2461** (upper) or **Rac1/cdc42 antibody** (lower).



Western blot analysis of extracts from mouse and rat brain tissue using **RhoB Antibody #2098**.



Western blot analysis of extracts from various cell types using **RhoC (D40E4) XP™ Rabbit mAb #3430**.



Western blot analysis of extracts from various cell types using **Rac1/2/3 (L129) Antibody #2467**.

## Western Immunoblotting Protocol (Primary Ab 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<sup>®</sup>-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<sup>®</sup> 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 cm<sup>2</sup>) 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<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> 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<sup>®</sup> incubation and declines over the following 2 hours.