

#4938 Store at -20°C

Rap1A/Rap1B Antibody



✓ 100 µl
(10 Western mini-blot)

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

Entrez-Gene ID # 5906, 5908
Swiss-Prot Acc. # P62834, P61224

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

Background: Rap1 and Rap2 belong to the Ras subfamily of small GTPases and are activated by a wide variety of stimuli through integrins, receptor tyrosine kinases (RTKs), G-protein coupled receptors (GPCR), death domain associated receptors (DD-R) and ion channels (1,2). Like other small GTPases, Rap activity is stimulated by guanine nucleotide exchange factors (GEF) and inactivated by GTPase activating proteins (GAP). A wide variety of Rap GEFs have been identified: C3G connects Rap1 with RTKs through adaptor proteins such as Crk, Epacs (or cAMP-GEFs) transmit signals from cAMP, and CD-GEFs (or CalDAG-GEFs) convey signals from either or both Ca²⁺ and DAG (1). Rap1 primarily regulates multiple integrin-dependent processes such as morphogenesis, cell-cell adhesion, hematopoiesis, leukocyte migration and tumor invasion (1,2). Rap1 may also regulate proliferation, differentiation and survival through downstream effectors including B-Raf, PI3K, RalGEF and phospholipases (PLCs) (1-4). Rap1 and Rap2 are not functionally redundant as they perform overlapping but distinct functions (5). Recent research indicates that Rap2 regulates Dsh subcellular localization and is required for Wnt signaling in early development (6).

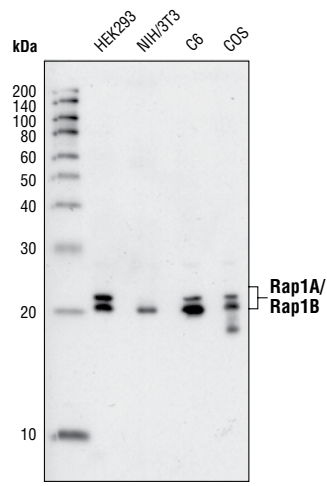
Specificity/Sensitivity: Rap1 Antibody detects endogenous levels of total Rap1A and Rap1B protein. This antibody does not cross-react with Rap2 and other Ras-related proteins.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues surrounding Gln130 of human Rap1A. Antibodies are purified by protein A and peptide affinity chromatography.

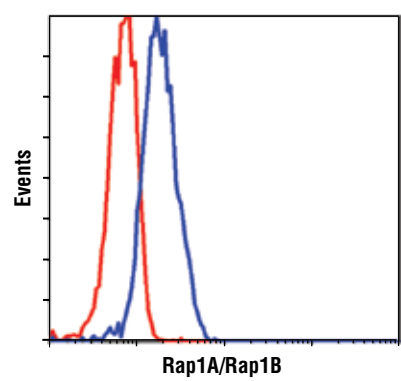
Background References:

- (1) Bos, J. et al. (2001) *Nat. Rev. Mol. Cell Biol.* 2, 369-377.
- (2) Caron, E. (2003) *J. Cell Sci.* 116, 435-440.
- (3) Song, C. et al. (2002) *Oncogene* 21, 8105-8113.
- (4) Rong, R. et al. (2003) *J. Biol. Chem.* 278, 52497-52503.
- (5) Taira, K. et al. (2004) *J. Biol. Chem.* 279, 49488-49496.
- (6) Choi, S. and Han, J. (2005) *EMBO J.* 24, 985-996.

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.



Western blot analysis of extracts from HEK293, NIH/3T3, C6 and COS cells, using Rap1A/Rap1B Antibody.



Flow cytometric analysis of untreated A431 cells, using Rap1A/Rap1B antibody (blue) compared to a nonspecific negative control antibody (red).

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
 Flow Cytometry 1:25

Companion Products:
 Rap1A/Rap1B (26B4) Rabbit mAb #2399
 Ras Antibody #3965
 R-Ras Antibody #3962
 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 #7727
 20X LumiGLO[®] Reagent and 20X Peroxide #7003

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.

Flow Cytometry Protocol for Intracellular Staining Using Conjugated Secondary Antibodies

A Solutions and Reagents

1. **1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 800 mL distilled water (dH_2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
2. Formaldehyde (methanol free)
3. **Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100mL 1X PBS. Store at 4°C

B Fixation

1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
3. Fix for 10 minutes at 37°C.
4. Chill tubes on ice for 1 minute.

C Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 minutes on ice.
3. Proceed with staining or store cells at -20°C in 90% methanol.

D Staining Using Unlabeled Primary and Conjugated Secondary Antibodies

NOTE: Allow for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemacytometer or alternative method.

1. Aliquot 0.5-1x10⁶ cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation. Repeat.
3. Resuspend cells in 100 μl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add the primary antibody at the appropriate dilution to the assay tubes (see individual antibody data sheet for the appropriate dilution).
6. Incubate for 30-60 minutes at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in fluorochrome-conjugated secondary antibody*, diluted in Incubation Buffer according to the manufacturer's recommendations.
9. Incubate for 30 minutes at room temperature.
10. Rinse as before in Incubation Buffer by centrifugation.
11. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.

*Recommended Secondary Antibodies from Invitrogen.

A-11070 Alexa Fluor® 488 F(ab')₂ fragment of goat anti-rabbit IgG (H+L) (1:1000 dilution)

A-11017 Alexa Fluor® 488 F(ab')₂ fragment of goat anti-mouse IgG (H+L) (1:1000 dilution)