

Rab Family Antibody Sampler Kit



1 Kit
 (5 x 40 µl)

Orders ■ 877-616-CELL (2355)
 orders@cellsignal.com
Support ■ 877-678-TECH (8324)
 info@cellsignal.com
Web ■ www.cellsignal.com

New 05/09

**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
Rab4 Antibody	2167	40 µl	25 kDa	Rabbit IgG
Rab5 Antibody	2143	40 µl	25 kDa	Rabbit IgG
Rab7 Antibody	2094	40 µl	23 kDa	Rabbit IgG
Rab9 Antibody	2095	40 µl	23 kDa	Rabbit IgG
Rab11 Antibody	3539	40 µl	25 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.

Description: The Rab Family Antibody Sampler Kit provides an economical means to evaluate the presence and status of Rab proteins in cells. This kit provides enough primary and secondary antibodies to perform four western mini-blot experiments per primary antibody.

Background: Rab family proteins are GTPases and members of the Ras superfamily of monomeric G proteins. These membrane-associated proteins are involved in many aspects of vesicle-mediated transport, taking part in the initial vesicle formation, transport of vesicles along the cytoskeleton, and eventual fusion of vesicle and target membranes. Rab4 is localized at early endosomes/recycling endosomes and functions as a key regulator for sorting/recycling of membrane and proteins (1,2). Both Rab4A and Rab4B isoforms are localized to similar cellular compartments and are believed to have similar functions (4). Rab4 interacts with several Rab4 effectors in a complex on a special endosome site to promote membrane/protein recycling (1,3). Rab5 is localized to the plasma membrane and early endosome and functions as a key regulator of vesicle trafficking during early endocytosis (1). The conformational change between Rab5-GTP and Rab5-GDP is essential for its biological function as a rate-limiting regulator at multiple steps during endocytosis (1,5). Similar to Rab4, Rab5 also interacts with specific Rab5 effectors on a specialized endosomal Rab domain to promote recycling between endosome and the plasma membrane (1,5,6). Both Rab7 and Rab9 are located in late endosomes but exert different functions. Rab7 associates with the RIPL effector protein to control membrane trafficking from early to late endosome and to lysosomes (7,8). Rab7 also helps to regulate growth receptor endocytic trafficking and degradation, and maturation of phagosome and autophagic vacuoles (8-11). Rab9 interacts with its effector proteins p40 and TIP47 (12,13) to promote the MPR (mannose 6-phosphate receptor)-associated lysosomal enzyme transport between late endosomes and the trans Golgi network (14,15). Rab11 (isoforms Rab11a and Rab11b) functions as a key regulator

in the recycling of perinuclear, plasma membrane and Golgi compartment endosomes (16,17). Despite some overlap, distinct differences exist between Rab11a and Rab11b in both their cellular distribution and functional roles. Rab11a is ubiquitously expressed while Rab11b is found mainly in the heart and brain (18,19). Like other Rab proteins, Rab11 functions when associated with Rab11 family interacting proteins (FIPs). The three distinct classes of Rab11 FIPs all share a conserved carboxy-terminal Rab-binding domain that allows Rab-FIP protein interaction. When bound together, these proteins are thought to regulate membrane-associated protein sorting (20,21).

Specificity/Sensitivity: Each antibody in the Rab Family Antibody Sampler Kit detects endogenous levels of its target protein. The Rab11 antibody detects endogenous levels of total Rab11 protein, including isoforms Rab11a and Rab11b.

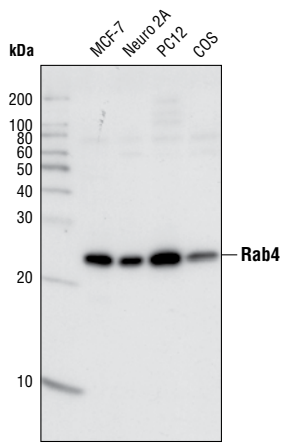
Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic peptides (KLH-coupled) corresponding to residues surrounding Asp128 of human Rab4 protein, Gly190 of human Rab5 protein, Asp193 of human Rab7, Asn184 of human Rab9, and Arg30 of human Rab11 protein. Antibodies are purified by protein A and peptide affinity chromatography.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. *Do not aliquot the antibodies.*

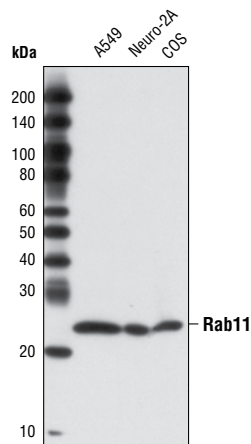
Recommended Antibody Dilutions:
 Western blotting 1:1000

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

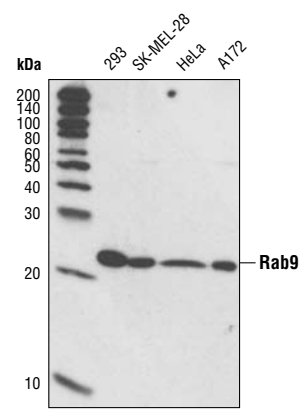
Background References:
 (1) Zerial, M. and McBride, H. (2001) *Nat Rev Mol Cell Biol* 2, 107-17.
 (2) van der Sluijs, P. et al. (1992) *Cell* 70, 729-40.
 (3) Deneka, M. et al. (2003) *EMBO J* 22, 2645-57.
 (4) Krawczyk, M. et al. (2007) *Nucleic Acids Res* 35, 595-605.
 (5) van der Blik, A.M. (2005) *Nat Cell Biol* 7, 548-50.
 (6) Haas, A.K. et al. (2005) *Nat Cell Biol* 7, 887-93.
 (7) Feng, Y. et al. (1995) *J Cell Biol* 131, 1435-52.
 (8) Méresse, S. et al. (1995) *J Cell Sci* 108 (Pt 11), 3349-58.
 (9) Ceresa, B.P. and Bahr, S.J. (2006) *J Biol Chem* 281, 1099-106.
 (10) Jäger, S. et al. (2004) *J Cell Sci* 117, 4837-48.
 (11) Méresse, S. et al. (1999) *EMBO J* 18, 4394-403.
 (12) Díaz, E. et al. (1997) *J Cell Biol* 138, 283-90.
 (13) Barbero, P. et al. (2002) *J Cell Biol* 156, 511-8.
 (14) Lombardi, D. et al. (1993) *EMBO J* 12, 677-82.
 (15) Riederer, M.A. et al. (1994) *J Cell Biol* 125, 573-82.
 (16) Ullrich, O. et al. (1996) *J Cell Biol* 135, 913-24.
 (17) Chen, W. et al. (1998) *Mol Biol Cell* 9, 3241-57.
 (18) Lapierre, L.A. et al. (2003) *Exp Cell Res* 290, 322-31.
 (19) Khvotchev, M.V. et al. (2003) *J Neurosci* 23, 10531-9.
 (20) Junutula, J.R. et al. (2004) *J Biol Chem* 279, 33430-7.
 (21) Hales, C.M. et al. (2001) *J Biol Chem* 276, 39067-75.



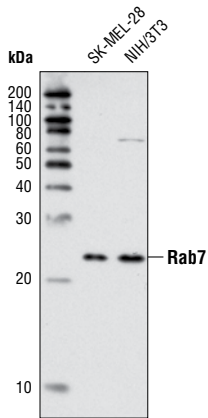
Western blot analysis of extracts from various cell types using **Rab4 Antibody #2167**.



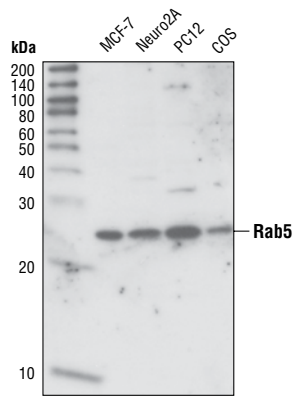
Western blot analysis of extracts from A549, Neuro-2A and COS cells using **Rab11 Antibody #3539**.



Western blot analysis of extracts from various cell types using **Rab9 Antibody #2095**.



Western blot analysis of extracts from SK-MEL-28 and NIH/3T3 cells using **Rab7 Antibody #2094**.



Western blot analysis of extracts from MCF-7, Neuro2A, PC12 and COS cells, using **Rab5 Antibody #2143**.

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.