

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Anti-Rab8

produced in rabbit, IgG fraction of antiserum

Product Number R5530

Product Description

Anti-Rab8 is produced in rabbit using as immunogen a synthetic peptide corresponding to a fragment of human Rab8A (GeneID: 4218), with an added cysteine, conjugated to KLH. The corresponding sequence differs by 2 amino acids in rat and mouse. Whole antiserum is purified using protein A immobilized on agarose to provide the IgG fraction of antiserum.

Anti-Rab8 recognizes human, rat, and mouse Rab8A. The antibody can be used in several techniques including immunoblotting (~24 kDa) and immunofluorescence staining. Detection of the Rab8 band by immunoblotting is specifically inhibited by the immunizing peptide.

Rab8 is a member of the Rab family of small guanosine triphosphatases (GTPases). The Rab family belongs to the Ras superfamily of small GTPases. Rab GTPases are central regulators of membrane trafficking between the different subcellular compartments of the eukaryotic cell. Their regulatory capacity depends on their ability to cycle between the GDP-bound inactive and GTP-bound active states. Conversion from one state to the other is regulated by GDP/GTP exchange factors (GEFs), GDP dissociation inhibitors (GDIs), and GTPase-activating proteins (GAPs). 1,2

Activation of a Rab protein is coupled to its association with intracellular membranes, allowing it to recruit downstream effector proteins to the cytoplasmic surface of a subcellular compartment.3 Through their effector proteins, Rab GTPases regulate vesicle formation, actin- and tubulin-dependent vesicle movement, and membrane fusion. 1 Rab proteins contain conserved regions involved in guanine-nucleotide binding, and hypervariable COOH-terminal domains with a cysteine motif, implicated in subcellular targeting. Posttranslational modification of the cysteine motif with one or two geranylgeranyl groups is essential for the membrane association and correct intracellular localization of Rab proteins.3 Each Rab shows a characteristic subcellular distribution.4 Therefore, antibodies to Rab proteins may serve as useful tools for studying subcellular localization and membrane organization.

Rab8 localizes to the TGN, recycling endosomes, vesicular and tubular structures in the cytosol, membrane protrusions, and plasma membrane. 5-7 Rab8 is involved in several transport pathways including vesicular traffic between the TGN and the basolateral plasma membrane in epithelial cells, transport of proteins to the dendritic surface in neurons, cell surface protrusion formation, actin-dependent movement of melanosomes, cell-cell adhesion, and apical protein localization in intestinal cells. 5,7-11 Rab8 interacts with Rab8ip (germinal center kinase), synaptotagmin-like protein 1 (JFC1/Slp1), optineurin and junctional Rab13binding protein (JRAB /MICAL-L2). The Rab8-JRAB /MICAL-L2 complex mediates the recycling of Ecadherin to the plasma membrane and the assembly of adherens junctions.7

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2–8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a working dilution of 1:250-1:500 is recommended using a whole extract of human Jurkat cells. It is highly recommended to use freshly prepared extracts.

Immunofluorescence: a working antibody dilution of 1:100-1:200 is recommended using mouse 3T3 and rat NRK cells fixed and permeabilized with 4% paraformaldehyde followed by 0.1% and 0.4% saponin, respectively.

<u>Note</u>: In order to obtain best results in various techniques and preparations, it is recommended to determine optimal working dilutions by titration.

References

- Stenmark, H., and Olkkonen, V.M., *Genome Biol.*, 2, 3007.1-3007.7 (2001).
- 2. Takai, Y., et al., *Physiol. Rev.*, **81**, 153-208 (2001).
- 3. Ali, B.R., et al., J. Cell Sci., 117, 6401-6412 (2004).

- 4. Zerial, M., and McBride, H., *Nature Rev. Mol. Cell Biol.*, **2**, 107-117 (2001).
- 5. Huber, L.A., et al., J. Cell Biol., 123, 35-45 (1993).
- 6. Ang, A.L., et al., J. Cell Biol., 163, 339-350 (2003).
- 7. Yamamura, R., et al., *Mol. Biol. Cell*, **19**, 971-983 (2008).
- 8. Huber, L.A., et al., J. Cell Biol., 123, 47-55 (1993).
- Hattula, K., et al., J. Cell Sci., 119, 4866-4877 (2006).
- Chabrillat, M.L., et al., Mol. Biol. Cell, 16, 1640-1650 (2005).
- 11. Sato, T., et al., *Nature*, **448**, 366-369 (2007).

VS,ST,KAA,PHC,MAM 01/19-1