10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

Anti-Rab5/RAB5A Antibody Picoband™ (monoclonal, 3E9)

Catalog # ABO14833

Product Information

Application	WB, IF, ICC, FC
Primary Accession	P20339
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Rab5/RAB5A Antibody Picoband™ (monoclonal, 3E9) . Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	5868
Other Names	Ras-related protein Rab-5A, 3.6.5.2, RAB5A, RAB5
Calculated MW	23659
Application Details	Western blot, 0.1-0.5 □g/ml Immunocytochemistry/Immunofluorescence, 2 □g/ml Flow Cytometry, 1-3 □g/1x10^6 cells
Subcellular Localization	Early endosome membrane. Lipid-anchor. Endosome membrane. Cytosol. Cell membrane. Cytoplasmic side. Melanosome. Cytoplasmic vesicle. Ruffle. Membrane. Phagosome membrane.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Clone Names	Clone: Clone: 3E9
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human Rab5, different from the related mouse and rat sequences by three amino acids.
Cross Reactivity	No cross-reactivity with other proteins.
Storage	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six

months. Avoid repeated freeze-thaw cycles.

Protein Information

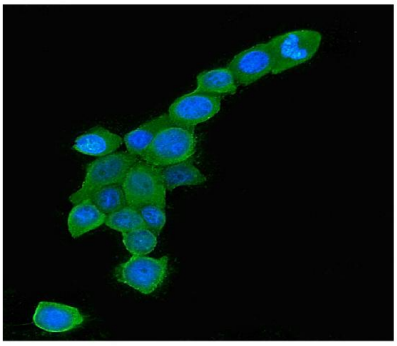
Name	RAB5A (HGNC:9783)
Synonyms	RAB5
Function	The small GTPases Rab are key regulators of intracellular membrane trafficking, from the formation of transport vesicles to their fusion with membranes. Rabs cycle between an inactive GDP-bound form and an active GTP-bound form that is able to recruit to membranes different sets of downstream effectors directly responsible for vesicle formation, movement, tethering and fusion. RAB5A is required for the fusion of plasma membranes and early endosomes and involved in early endocytic trafficking (PubMed:10818110, PubMed:14617813, PubMed:15378032, PubMed:16086013, PubMed:16410077, PubMed:17562788). Required for EEA1 recruitment to early endosomes (PubMed:16086013, PubMed:17562788). Recruits FERRY complex to early endosomes, where FERRY links early endosomes with a subgroup of mRNAs to enable mRNA intracellular distribution (PubMed:37267906). Recruits RABEP1/Rabaptin- 5 effector to early endosomes, thereby promoting endocytic membrane fusion (By similarity). Required for EGF and transferrin endocytosis and trafficking through early endosomes (PubMed:16086013, PubMed:17562788). Contributes to the regulation of filopodia extension (PubMed:14978216). Required for the exosomal release of SDCBP, CD63, PDCD6IP and syndecan (PubMed:22660413). Regulates maturation of apoptotic cell-containing phagosomes, probably downstream of DYN2 and PIK3C3 (By similarity).
Cellular Location	Cell membrane; Lipid-anchor; Cytoplasmic side. Early endosome membrane; Lipid-anchor. Melanosome Cytoplasmic vesicle. Cell projection, ruffle {ECO:0000250|UniProtKB:P18066}. Membrane. Cytoplasm, cytosol. Cytoplasmic vesicle, phagosome membrane {ECO:0000250|UniProtKB:Q9CQD1}. Endosome membrane Note=Enriched in stage I melanosomes (PubMed:17081065). Alternates between membrane-bound and cytosolic forms (Probable) {ECO:0000269|PubMed:17081065, ECO:0000305}

Background

RAB5A (Ras-associated protein RAB5A), also called RAB5, is a protein that in humans is encoded by the RAB5A gene. RAB5 is a rate-limiting component of the machinery regulating the kinetics of membrane traffic in the early endocytic pathway. The RAB5A gene is mapped on 3p24.3. RAB5 is indispensable for a form of receptor tyrosine kinase-induced actin remodeling called circular ruffling.It signals to the actin cytoskeleton through RNTRE, a RAB5-specific GTPase-activating protein (GAP).RAB5 activity on phagosome membranes began to increase on disassembly of the actin coat encapsulating phagosomes. In addition, RAB5 activation is either continuous or repetitive for up to 10 minutes, but it ends before the collapse of engulfed apoptotic cells. Expression of a dominant-negative mutant of RAB5 delayed this collapse of apoptotic thymocytes, showing a role for RAB5 in phagosome maturation.

Images

Figure 1. IF analysis of RAB5A using anti-RAB5A antibody (M01891-1).
RAB5A was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval was performed



using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-RAB5A Antibody (M01891-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

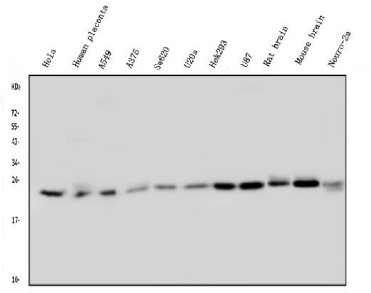


Figure 2. Western blot analysis of Rab5/RAB5A using anti-Rab5/RAB5A antibody (M01891-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates,
Lane 2: human placenta tissue lysates,
Lane 3: human A549 whole cell lysates.
Lane 4: human A375 whole cell lysates,
Lane 5: human SW620 whole cell lysates,
Lane 6: human U20S whole cell lysates,
Lane 7: human HEK293 whole cell lysates,
Lane 8: human U87 whole cell lysates,
Lane 9: rat brain tissue lysates,
Lane 10: mouse brain tissue lysates,
Lane 11: mouse Neuro-2a whole cell lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-Rab5/RAB5A antigen affinity purified monoclonal antibody (Catalog # M01891-1) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for Rab5/RAB5A at approximately 24KD. The expected band size for Rab5/RAB5A is at 24KD.

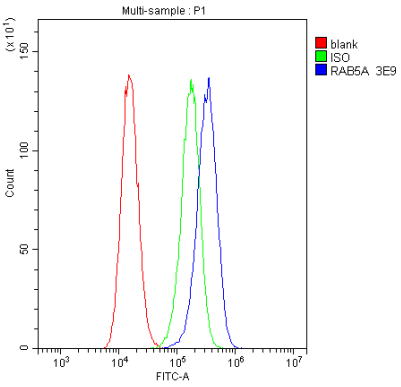


Figure 3. Flow Cytometry analysis of A431 cells using anti-Rab5/RAB5A antibody (M01891-1).
Overlay histogram showing A431 cells stained with M01891-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Rab5/RAB5A Antibody (M01891-1, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.