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Anti-RALBP1 Antibody Picoband™ (monoclonal, 2E11)

Catalog # ABO15060

Product Information

Application	WB, FC
Primary Accession	Q15311
Host	Mouse
Isotype	Mouse IgG2a
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-RALBP1 Antibody Picoband™ (monoclonal, 2E11) . Tested in Flow Cytometry, WB applications. This antibody reacts with Human.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	10928
Other Names	RalA-binding protein 1, 76 kDa Ral-interacting protein, Dinitrophenyl S-glutathione ATPase, DNP-SG ATPase, 7.6.2.2, 7.6.2.3, Ral-interacting protein 1, RALBP1 (HGNC:9841)
Calculated MW	76063
Application Details	Western blot, 0.25-0.5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 2E11
Immunogen	E. coli-derived human RALBP1 recombinant protein (Position: K239-Q506).
Purification	Immunogen affinity purified.
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	RALBP1 (HGNC:9841)

Function	Multifunctional protein that functions as a downstream effector of RALA and RALB (PubMed:7673236). As a GTPase-activating protein/GAP can inactivate CDC42 and RAC1 by stimulating their GTPase activity (PubMed:7673236). As part of the Ral signaling pathway, may also regulate ligand-dependent EGF and insulin receptors-mediated endocytosis (PubMed:10910768, PubMed:12775724). During mitosis, may act as a scaffold protein in the phosphorylation of EPSIN/EPN1 by the mitotic kinase cyclin B-CDK1, preventing endocytosis during that phase of the cell cycle (PubMed:12775724). During mitosis, also controls mitochondrial fission as an effector of RALA (PubMed:21822277). Recruited to mitochondrion by RALA, acts as a scaffold to foster the mitotic kinase cyclin B-CDK1-mediated phosphorylation and activation of DNM1L (PubMed:21822277).
Cellular Location	Cell membrane; Peripheral membrane protein. Cytoplasm, cytosol Cytoplasm, cytoskeleton, spindle pole {ECO:0000250|UniProtKB:Q62796} Nucleus. Mitochondrion. Note=Cytosolic protein that transiently associates with the mitotic spindle poles in early prophase, and dissociates from them after completion of mitosis (By similarity) Targeted to the plasma membrane through its interaction with RALB, directed by FGF signaling. Docking on the membrane is required to transduce the Ral signal (By similarity). Recruited by RALA to the mitochondrion during mitosis where it regulates mitochondrial fission (PubMed:21822277). Nuclear localization is cell cycle dependent while membrane localization is seen in adherent cells (PubMed:22319010). The region involved in membrane association could form transmembrane domains and expose a part of the protein extracellularly (Probable) {ECO:0000250|UniProtKB:Q62796, ECO:0000250|UniProtKB:Q9PT60, ECO:0000269|PubMed:21822277, ECO:0000269|PubMed:22319010, ECO:0000305|PubMed:15610018}
Tissue Location	Expressed ubiquitously but at low levels. Shows a strong expression in the erythrocytes.

Background

RalA-binding protein 1 is a protein that in humans is encoded by the RALBP1 gene. Small G proteins, such as RAL, have GDP-bound inactive and GTP-bound active forms, which shift from the inactive to the active state through the action of RALGDS, which in turn is activated by RAS. RALBP1 plays a role in receptor-mediated endocytosis and is a downstream effector of the small GTP-binding protein RAL. RALBP1 is also the dominant transporter of lipid peroxidation-derived glutathione conjugates and participates in several mitotic events, including inactivation of endocytosis and separation and polar movement of centrioles and appropriate distribution of mitochondria to daughter cells following mitosis.

Images

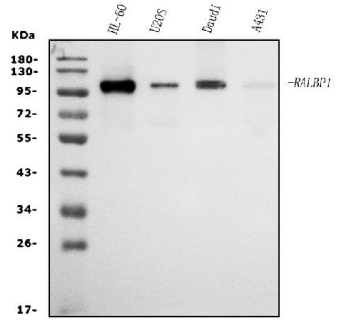


Figure 1. Western blot analysis of RALBP1 using anti-RALBP1 antibody (M01403-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours.
The sample well of each lane was loaded with 30ug of sample under reducing conditions.
Lane 1: human HL-60 whole cell lysates,
Lane 2: human U20S whole cell lysates,
Lane 3: human Daudi whole cell lysates,
Lane 4: human A431 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-RALBP1 antigen affinity purified monoclonal antibody (Catalog # M01403-2) 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 RALBP1 at approximately 95KD. The expected band size for RALBP1 is at 95KD.

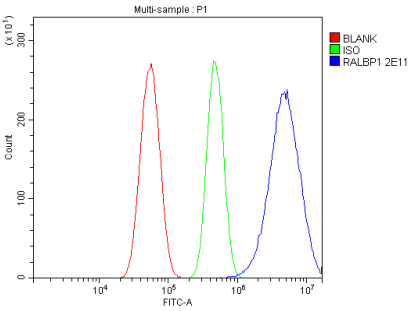


Figure 2. Flow Cytometry analysis of U87 cells using anti-RALBP1 antibody (M01403-2).
Overlay histogram showing U87 cells stained with M01403-2 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-RALBP1 Antibody (M01403-2, 1 µg/1x10^6 cells) for 30 min at 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.

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