10320 Camino Santa Fe, Suite G
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Anti-RALBP1 Antibody Picoband™ (monoclonal, 2F9D3)

Catalog # ABO15126

Specification

Anti-RALBP1 Antibody Picoband™ (monoclonal, 2F9D3) - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	Q15311
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-RALBP1 Antibody Picoband™ (monoclonal, 2F9D3) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.

Reconstitution
Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.

Anti-RALBP1 Antibody Picoband™ (monoclonal, 2F9D3) - 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
95 kDa KDa

Application Details
Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat
 Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Human, Mouse
 Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human
 Flow Cytometry, 1-3 µg/1x10^6 cells, Human

Contents
Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.

Immunogen
E. coli-derived human RALBP1 recombinant protein (Position: K239-Q506).

Purification
Immunogen affinity purified.

Storage	**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 freezing and thawing.

Anti-RALBP1 Antibody Picoband™ (monoclonal, 2F9D3) - 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.

Anti-RALBP1 Antibody Picoband™ (monoclonal, 2F9D3) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

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Anti-RALBP1 Antibody Picoband™ (monoclonal, 2F9D3) - Images

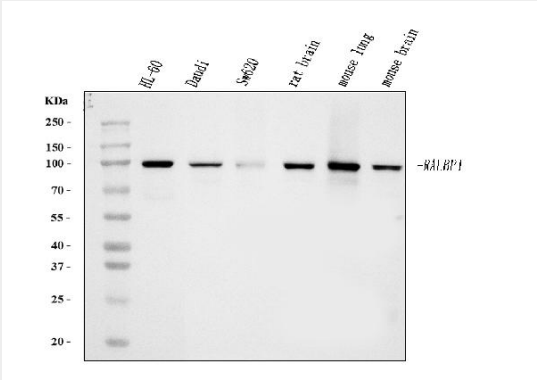

Figure 1. Western blot analysis of RALBP1 using anti-RALBP1 antibody (M01403-4).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human HL-60 whole cell lysates,
Lane 2: human Daudi whole cell lysates,
Lane 3: human SW620 whole cell lysates,
Lane 4: rat brain tissue lysates,
Lane 5: mouse lung tissue lysates,
Lane 6: mouse brain tissue lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA 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 # M01403-4) 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 95 kDa. The expected band size for RALBP1 is at 95 kDa.

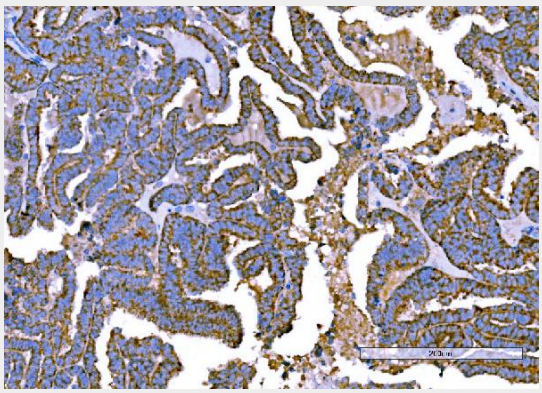

Figure 2. IHC analysis of RALBP1 using anti-RALBP1 antibody (M01403-4).
RALBP1 was detected in a paraffin-embedded section of human ovarian cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-RALBP1 Antibody (M01403-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

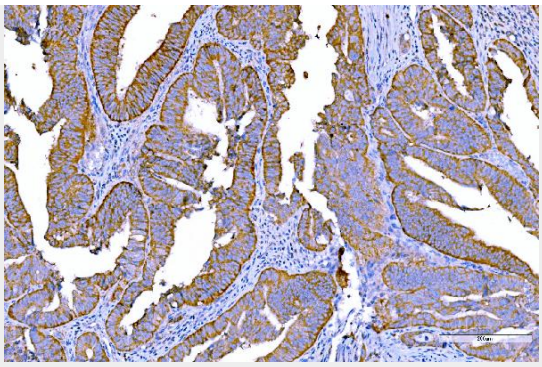

Figure 3. IHC analysis of RALBP1 using anti-RALBP1 antibody (M01403-4).
RALBP1 was detected in a paraffin-embedded section of human rectum adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-RALBP1 Antibody (M01403-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

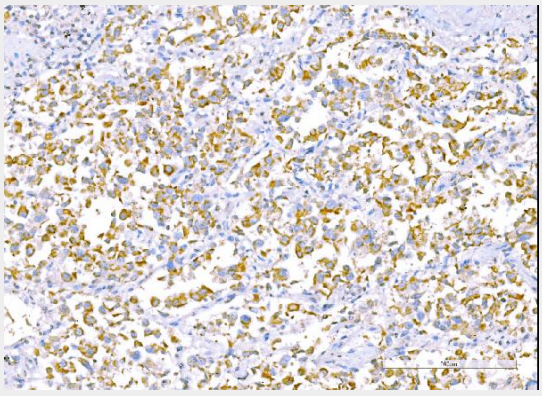

Figure 4. IHC analysis of RALBP1 using anti-RALBP1 antibody (M01403-4).
RALBP1 was detected in a paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-RALBP1 Antibody (M01403-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

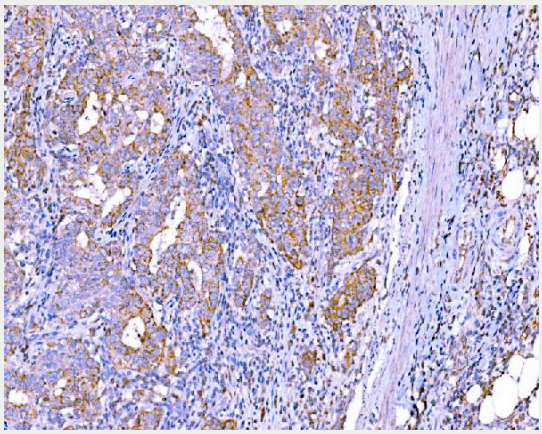

Figure 5. IHC analysis of RALBP1 using anti-RALBP1 antibody (M01403-4).
RALBP1 was detected in a paraffin-embedded section of human metaplasia of squamous cells of the renal pelvis tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-RALBP1 Antibody (M01403-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

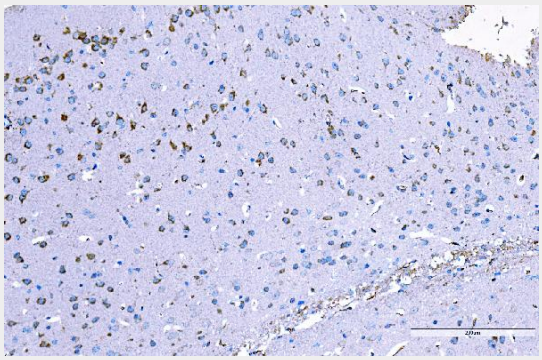

Figure 6. IHC analysis of RALBP1 using anti-RALBP1 antibody (M01403-4).
RALBP1 was detected in a paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-RALBP1 Antibody (M01403-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

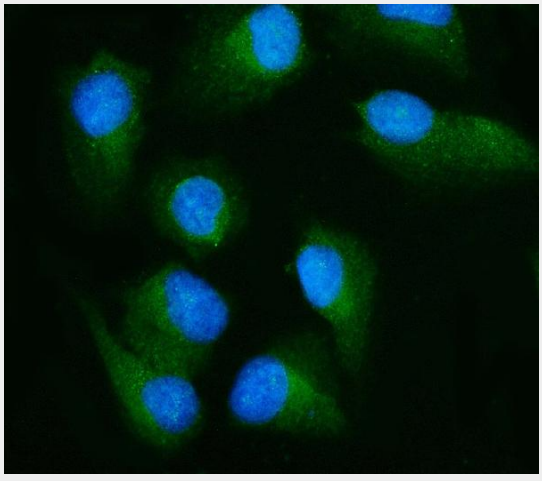

Figure 7. IF analysis of RALBP1 using anti-RALBP1 antibody (M01403-4).
RALBP1 was detected in an immunocytochemical section of U20S cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-RALBP1 Antibody (M01403-4) 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.

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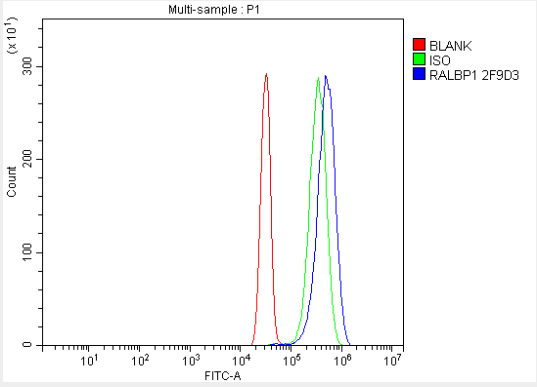

Figure 8. Flow Cytometry analysis of SiHa cells using anti-RALBP1 antibody (M01403-4). Overlay histogram showing SiHa cells stained with M01403-4 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-RALBP1 Antibody (M01403-4, 1 µg/1x10^6 cells) for 30 min 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.

Anti-RALBP1 Antibody Picoband™ (monoclonal, 2F9D3) - 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.