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Anti-Grp75 Antibody Picoband™ (monoclonal, 4I9)

Catalog # ABO15103

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P38646
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Grp75 Antibody Picoband™ (monoclonal, 4I9) . 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 □g/ml.

Additional Information

Gene ID	3313
Other Names	Stress-70 protein, mitochondrial, 75 kDa glucose-regulated protein, GRP-75, Heat shock 70 kDa protein 9, Heat shock protein family A member 9, Mortalin, MOT, Peptide-binding protein 74, PBP74, HSPA9 (HGNC:5244), GRP75, HSPA9B, mt-HSP70
Calculated MW	73680
Application Details	Western blot, 0.25-0.5 □g/ml, Human, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 □g/ml, Human Immunocytochemistry/Immunofluorescence, 5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
Clone Names	Clone: 4I9
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human Grp75, identical to the related mouse and rat sequences.
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.

Protein Information

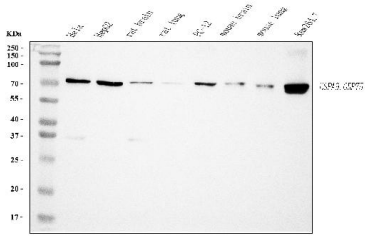
Name	HSPA9 (HGNC:5244)
Function	Mitochondrial chaperone that plays a key role in mitochondrial protein import, folding, and assembly. Plays an essential role in the protein quality control system, the correct folding of proteins, the re-folding of misfolded proteins, and the targeting of proteins for subsequent degradation. These processes are achieved through cycles of ATP binding, ATP hydrolysis, and ADP release, mediated by co-chaperones (PubMed:18632665, PubMed:25615450, PubMed:28848044, PubMed:30933555, PubMed:31177526). In mitochondria, it associates with the TIM (translocase of the inner membrane) protein complex to assist in the import and folding of mitochondrial proteins (By similarity). Plays an important role in mitochondrial iron-sulfur cluster (ISC) biogenesis, interacts with and stabilizes ISC cluster assembly proteins FXN, NFU1, NFS1 and ISCU (PubMed:26702583). Regulates erythropoiesis via stabilization of ISC assembly (PubMed:21123823, PubMed:26702583). Regulates mitochondrial calcium-dependent apoptosis by coupling two calcium channels, ITPR1 and VDAC1, at the mitochondria-associated endoplasmic reticulum (ER) membrane to facilitate calcium transport from the ER lumen to the mitochondria intermembrane space, providing calcium for the downstream calcium channel MCU, which releases it into the mitochondrial matrix (By similarity). Although primarily located in the mitochondria, it is also found in other cellular compartments. In the cytosol, it associates with proteins involved in signaling, apoptosis, or senescence. It may play a role in cell cycle regulation via its interaction with and promotion of degradation of TP53 (PubMed:24625977, PubMed:26634371). May play a role in the control of cell proliferation and cellular aging (By similarity). Protects against reactive oxygen species (ROS) (By similarity). Extracellular HSPA9 plays a cytoprotective role by preventing cell lysis following immune attack by the membrane attack complex by disrupting formation of the complex (PubMed:16091382).
Cellular Location	Mitochondrion. Nucleus, nucleolus. Cytoplasm. Mitochondrion matrix {ECO:0000250|UniProtKB:P48721}. Note=Found in a complex with HSPA9 and VDAC1 at the endoplasmic reticulum-mitochondria contact sites {ECO:0000250|UniProtKB:P48721}

Background

HSPA9 (heat shock 70kDa protein 9 (mortalin)), also known as GRP75, mot-2, mthsp75, PBP74, HSPA9B, MORTALIN or MORTALIN, PERINUCLEAR, is a highly conserved member of the HSP70 family of proteins. It functions as a chaperone in the mitochondria, cytoplasm, and centrosome. The HSPA9 gene is mapped to chromosome 5q31.2 based on an alignment of the HSPA9 sequence with the genomic sequence. Knockdown of HSPA9 in erythroid cultures was associated with an increased number of cells in the G0/G1 phase of the cell cycle and accelerated apoptosis. Knockdown of Hspa9 in mouse bone marrow cells, followed by transplantation into wildtype recipients, also resulted in loss of erythroid cell number. Haploinsufficiency for HSPA9 may contribute to abnormal hematopoiesis in myelodysplastic syndromes. This protein plays a role in the control of cell proliferation.

Images

Figure 1. Western blot analysis of Grp75 using anti-Grp75 antibody (M02561-2).
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 30 ug of



sample under reducing conditions.
Lane 1: human Hela whole cell lysates,
Lane 2: human HepG2 whole cell lysates,
Lane 3: rat brain tissue lysates,
Lane 4: rat lung tissue lysates,
Lane 5: rat PC-12 whole cell lysates,
Lane 6: mouse brain tissue lysates,
Lane 7: mouse lung tissue lysates,
Lane 8: mouse RAW264.7 whole cell 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-Grp75 antigen affinity purified monoclonal antibody (Catalog # M02561-2) 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 Grp75 at approximately 74 kDa. The expected band size for Grp75 is at 74 kDa.

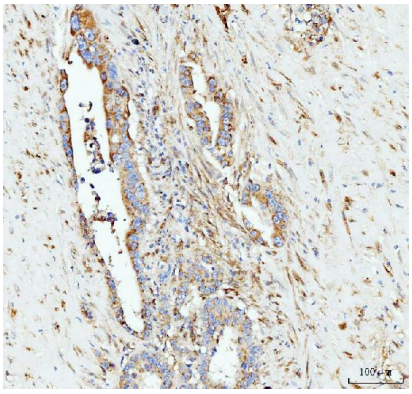


Figure 2. IHC analysis of Grp75 using anti-Grp75 antibody (M02561-2).
Grp75 was detected in a paraffin-embedded section of human appendiceal adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Grp75 Antibody (M02561-2) 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.

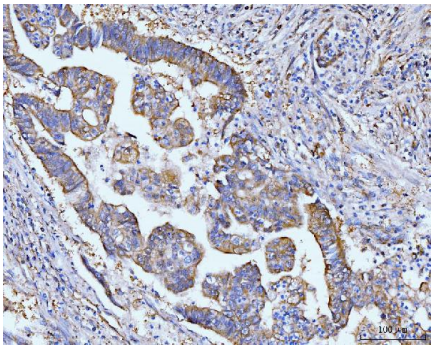
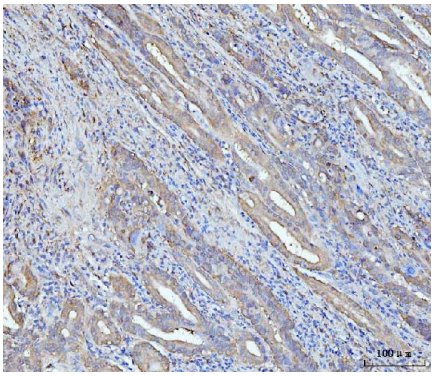


Figure 3. IHC analysis of Grp75 using anti-Grp75 antibody (M02561-2).
Grp75 was detected in a paraffin-embedded section of human gastric cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Grp75 Antibody (M02561-2) 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 Grp75 using anti-Grp75 antibody (M02561-2).
Grp75 was detected in a paraffin-embedded section of human gall bladder adenosquamous carcinoma 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-Grp75 Antibody (M02561-2) 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.

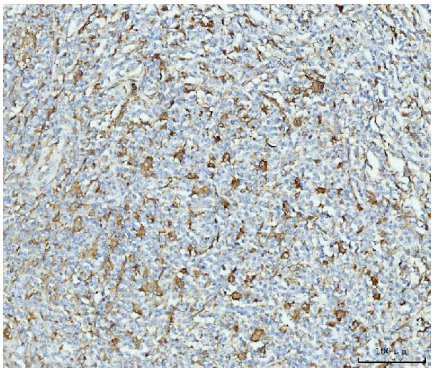


Figure 5. IHC analysis of Grp75 using anti-Grp75 antibody (M02561-2).
Grp75 was detected in a paraffin-embedded section of human lymphadenoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Grp75 Antibody (M02561-2) 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.

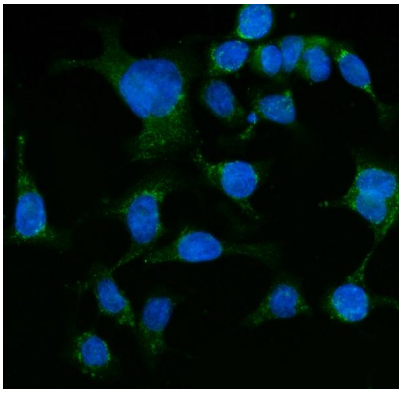


Figure 6. IF analysis of Grp75 using anti-Grp75 antibody (M02561-2).
Grp75 was detected in an immunocytochemical section of CACO-2 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-Grp75 Antibody (M02561-2) 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.

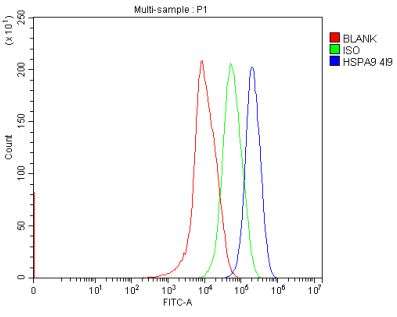


Figure 7. Flow Cytometry analysis of HepG2 cells using anti-Grp75 antibody (M02561-2).
Overlay histogram showing HepG2 cells stained with M02561-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Grp75 Antibody (M02561-2, 1 µg/1x10^6 cells) for 30 min 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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