

Anti-Grp75 Antibody Picoband™ (monoclonal, 4I9)
Catalog # ABO15103**Specification****Anti-Grp75 Antibody Picoband™ (monoclonal, 4I9) - 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 µg/ml.

Anti-Grp75 Antibody Picoband™ (monoclonal, 4I9) - 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](http://www.genenames.org/cgi-bin/gene_symbol_report?hgnc_id=5244)), GRP75, HSPA9B, mt-HSP70

Calculated MW

74 kDa KDa

Application Details

Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat
Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Human
Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human
Flow Cytometry, 1-3 µg/1x10⁶ cells, Human

Contents

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

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.

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

Name HSPA9 ([HGNC:5244](#))

Synonyms GRP75, HSPA9B, mt-HSP70

Function

Chaperone protein which plays an important role in mitochondrial iron-sulfur cluster (ISC) biogenesis. Interacts with and stabilizes ISC cluster assembly proteins FXN, NFS1 and ISCU (PubMed:26702583). Regulates erythropoiesis via stabilization of ISC assembly (PubMed:21123823, PubMed:26702583). 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). Molecular adapter that 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, thus providing calcium for the downstream calcium channel MCU that directly releases it into mitochondria matrix (By similarity).

Cellular Location

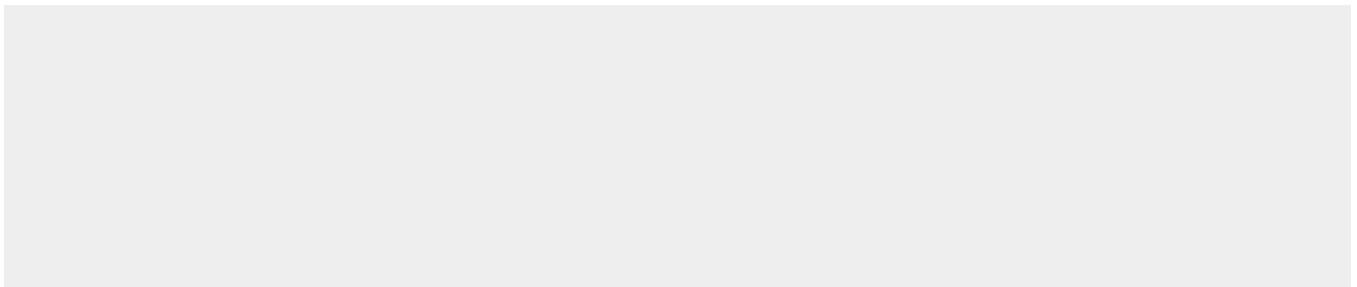
Mitochondrion. Nucleus, nucleolus. Cytoplasm. Mitochondrion matrix {ECO:0000250|UniProtKB:P48721}. Note=Found in a complex with HSPA9 and VDAC1 at the endoplasmic reticulum-mitochondria contact sites {ECO:0000250|UniProtKB:P48721}

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

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

- [Western Blot](#)
- [Blocking Peptides](#)
- [Dot Blot](#)
- [Immunohistochemistry](#)
- [Immunofluorescence](#)
- [Immunoprecipitation](#)
- [Flow Cytometry](#)
- [Cell Culture](#)

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



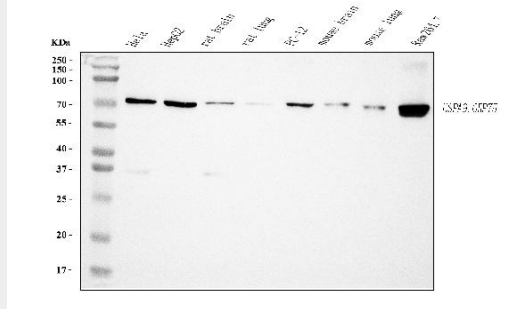


Figure 1. Western blot analysis of Grp75 using anti-Grp75 antibody (M02561-2).

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.

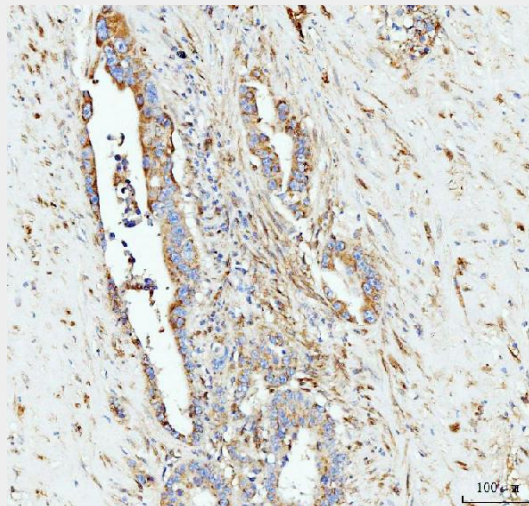


Figure 2. IHC analysis of Grp75 using anti-Grp75 antibody (M02561-2).

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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

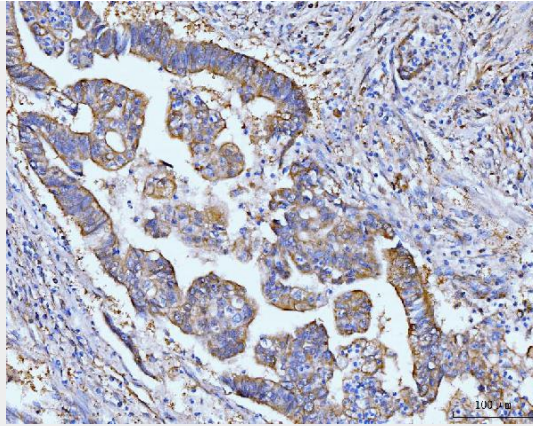


Figure 3. IHC analysis of Grp75 using anti-Grp75 antibody (M02561-2).

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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

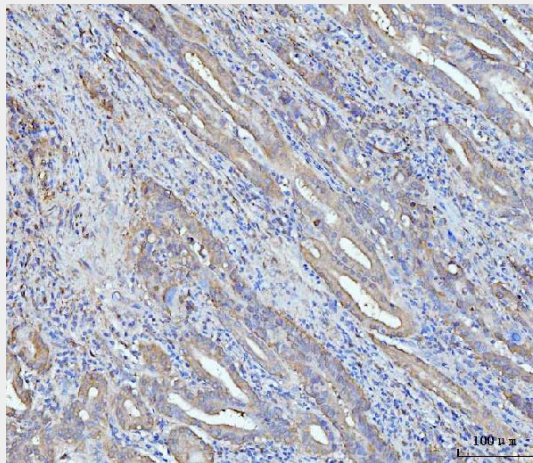


Figure 4. IHC analysis of Grp75 using anti-Grp75 antibody (M02561-2).

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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

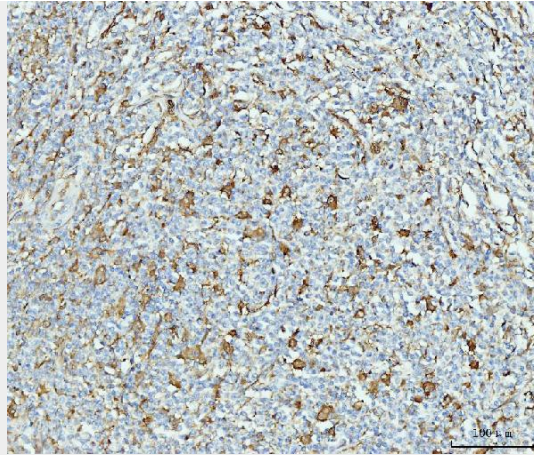


Figure 5. IHC analysis of Grp75 using anti-Grp75 antibody (M02561-2).

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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

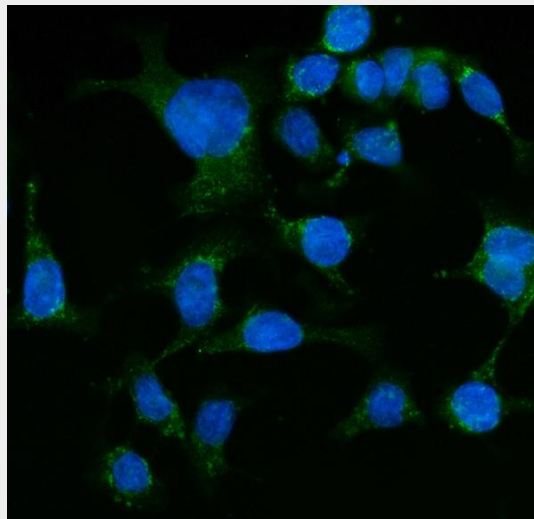


Figure 6. IF analysis of Grp75 using anti-Grp75 antibody (M02561-2).

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.

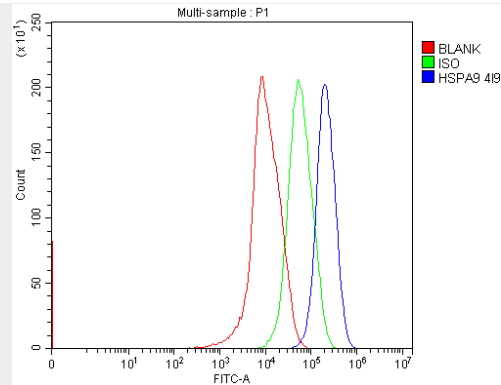


Figure 7. Flow Cytometry analysis of HepG2 cells using anti-Grp75 antibody (M02561-2). Overlay histogram showing HepG2 cells stained with M02561-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Grp75 Antibody (M02561-2, 1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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