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antibody and ELISA experts

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

Catalog Number: M02561-2

About HSPA9

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.

Overview

Product Name	Anti-Grp75 Antibody Picoband™ (monoclonal, 4I9)
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-Grp75 Antibody Picoband™ (monoclonal, 4I9) catalog # M02561-2. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Monoclonal 4I9
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
Storage Instructions	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.
Host	Mouse
Uniprot ID	P38646

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human Grp75, identical to the related mouse and rat sequences.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG1
Form	Lyophilized

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Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: Western blot, 0.25-0.5 ug/ml, Human, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 ug/ml, Human Immunocytochemistry/Immunofluorescence, 5 ug/ml, Human Flow Cytometry, 1-3 ug/1x10^6 cells, Human

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Anti-Grp75 Antibody Picoband™ (monoclonal, 4I9) (M02561-2) Images

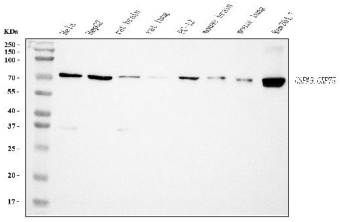

Figure 1. Western blot analysis of Grp75 using anti-Grp75 antibody (M02561-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human Hela whole 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 ug/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.

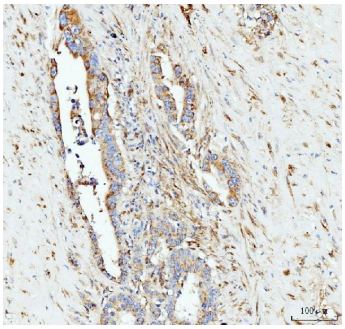

Figure 2. IHC analysis of Grp75 using anti-Grp75 antibody (M02561-2).
Grp75 was detected in a paraffin-embedded section of human appendiceal adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10%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

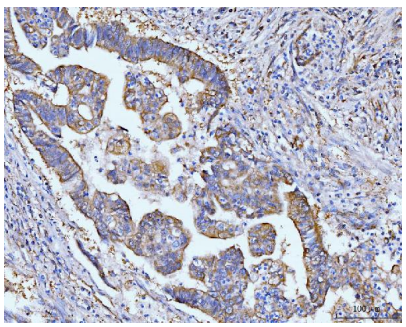

Figure 3. IHC analysis of Grp75 using anti-Grp75 antibody (M02561-2).
Grp75 was detected in a paraffin-embedded section of human gastric cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum.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

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

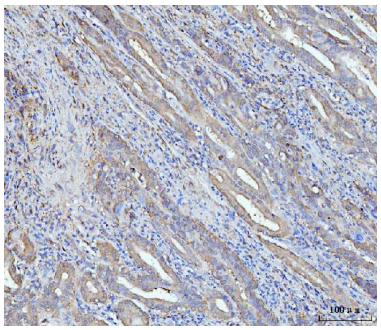

Figure 4. IHC analysis of Grp75 using anti-Grp75 antibody (M02561-2).
Grp75 was detected in a paraffin-embedded section of human gall bladder adenosquamous carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/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.

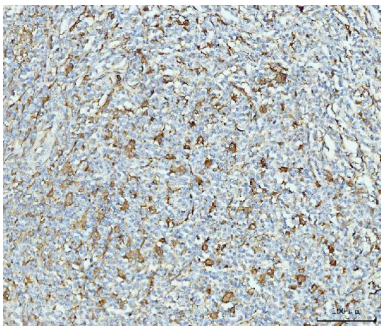

Figure 5. IHC analysis of Grp75 using anti-Grp75 antibody (M02561-2).
Grp75 was detected in a paraffin-embedded section of human lymphadenoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum.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

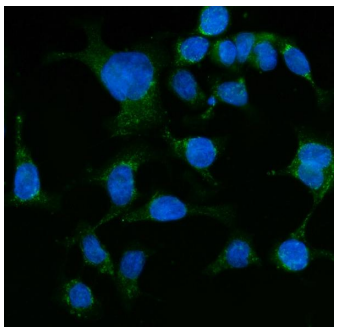

Figure 6. IF analysis of Grp75 using anti-Grp75 antibody (M02561-2).
Grp75 was detected in an immunocytochemical section of CACO-2 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated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

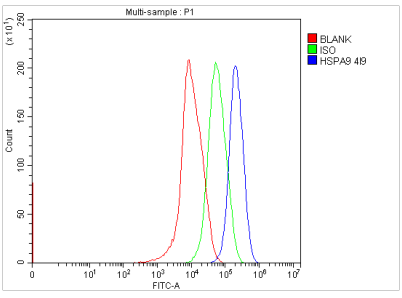

Figure 7. Flow Cytometry analysis of HepG2 cells using anti-Grp75 antibody (M02561-2).
Overlay histogram showing HepG2 cells stained with M02561-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Grp75 Antibody (M02561-2, 1 ug/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 ug/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 ug/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used

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as a control.

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