Product Datasheet

Grp75/HSPA9 Rabbit Polyclonal Antibody (orb315150)

Catalog Number	orb315150
Category	Antibodies
Description	Grp75/HSPA9 Rabbit Polyclonal Antibody
Target	Stress-70 protein, mitochondrial
Clonality	Polyclonal
Species/Host	Rabbit
Isotype	Rabbit IgG
Conjugation	Unconjugated
Reactivity	Human, Mouse, Rat
Form/Appearance	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
Buffer/Preservatives	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.01mg NaN3.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.
Purification	Immunogen affinity purified.
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human Grp75, identical to the related mouse and rat sequences.
UniProt ID	P38646

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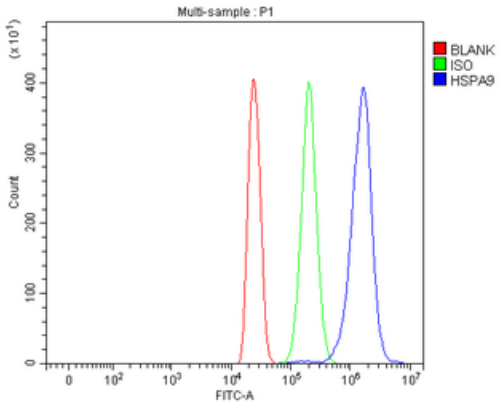
MW	75 kDa
Tested applications	FC, ICC, IF, IHC, IP, WB
Dilution range	Western blot, 0.1-0.5µg/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5µg/ml, Human Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Immunoprecipitation, 0.5-2 µg/ml, Human Flow Cytometry(Fixed), 1-3 µg/1x10^6 cells, Human
Specificity	No cross reactivity with other proteins.
Cross Reactivity	No cross-reactivity with other proteins
Antibody Type	Primary Antibody
Storage	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
Note	For research use only
Expiration Date	12 months from date of receipt.



Flow Cytometry analysis of HL-60 cells using anti-Grp75 antibody. Overlay histogram showing HL-60 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Grp75 Antibody (1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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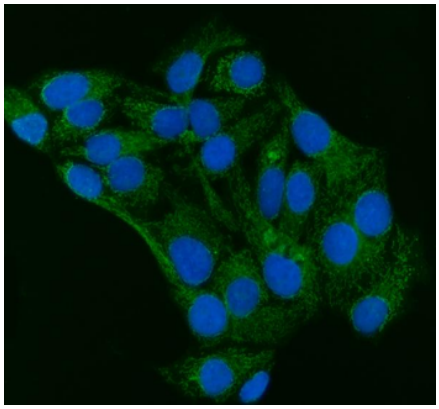
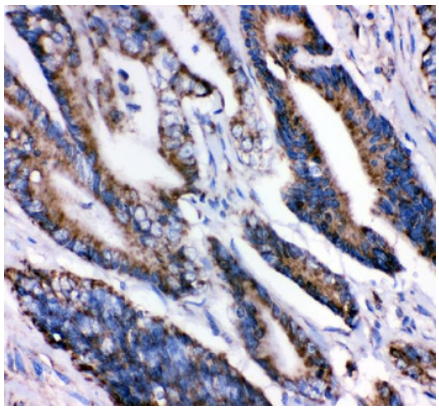
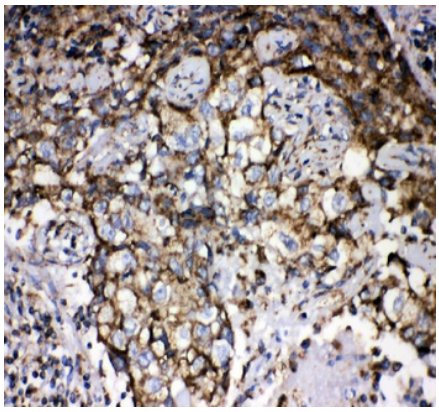


IF analysis of Grp75 using anti-Grp75 antibody. Grp75 was detected in an immunocytochemical section of Hela cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL rabbit anti-Grp75 Antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:500 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.



IHC analysis of Grp75 using anti-Grp75 antibody. Grp75 was detected in a paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-Grp75 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.



IHC analysis of Grp75 using anti-Grp75 antibody. Grp75 was detected in a paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-Grp75 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.

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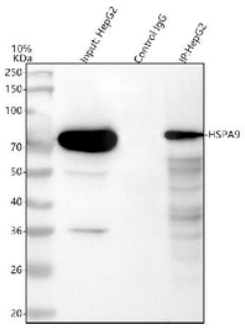
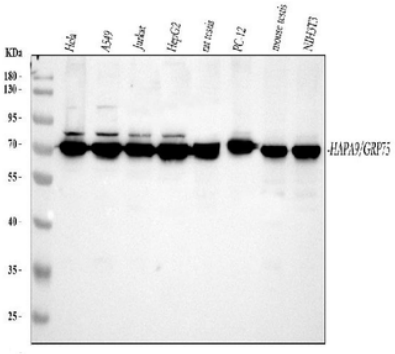


Immunoprecipitating Grp75 in HepG2 whole cell lysate. Western blot analysis of Grp75 using anti-Grp75 antibody. Lane 1: HepG2 whole cell lysates (30 ug), Lane 2: Rabbit control IgG instead of anti-Grp75 antibody in HepG2 whole cell lysate, Lane 3: anti-Grp75 antibody (2 µg) + HepG2 whole cell lysate (500 µg). After electrophoresis, proteins were transferred to a membrane. Then the membrane was incubated with rabbit anti-Grp75 antigen affinity purified polyclonal antibody at a dilution of 0.5 µg/mL and probed with a mouse anti-rabbit IgG-HRP secondary antibody. The signal is developed using ECL Plus Western Blotting Substrate. A specific band was detected for DDX5 at approximately 74 kDa. The expected band size for DDX5 is at 74 kDa.



Western blot analysis of Grp75 using anti-Grp75 antibody. 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 A549 whole cell lysates, Lane 3: human Jurkat whole cell lysates, Lane 4: human HepG2 whole cell lysates, Lane 5: rat testis tissue lysates, Lane 6: rat PC-12 whole cell lysates, Lane 7: mouse testis tissue lysates, Lane 8: mouse NIH/3T3 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 rabbit anti-Grp75 antigen affinity purified polyclonal antibody 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-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit 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.

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