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Anti-Hsp60/HSPD1 Antibody Picoband™ (monoclonal, 6G2)

Catalog # ABO14873

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P10809
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Hsp60/HSPD1 Antibody Picoband™ (monoclonal, 6G2) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	3329
Other Names	60 kDa heat shock protein, mitochondrial, 5.6.1.7, 60 kDa chaperonin, Chaperonin 60, CPN60, Heat shock protein 60, HSP-60, Hsp60, Heat shock protein family D member 1, HuCHA60, Mitochondrial matrix protein P1, P60 lymphocyte protein, HSPD1, HSP60
Calculated MW	61055
Application Details	Western blot, 0.1-0.5 □g/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml Immunocytochemistry/Immunofluorescence, 5 □g/ml Flow Cytometry, 1-3 □g/1x10^6 cells
Subcellular Localization	Mitochondrion matrix.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na_2HPO_4, 0.05mg NaN_3.
Clone Names	Clone: 6G2
Immunogen	E.coli-derived human Hsp60/HSPD1 recombinant protein (Position: A260-Q496). Human Hsp60 shares 97% amino acid (aa) sequence identity with both mouse and rat Hsp60.
Cross Reactivity	No cross-reactivity with other proteins.

Storage	Store 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 freeze-thaw cycles.

Protein Information

Name	HSPD1
Synonyms	HSP60
Function	Chaperonin implicated in mitochondrial protein import and macromolecular assembly. Together with Hsp10, facilitates the correct folding of imported proteins. May also prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions in the mitochondrial matrix (PubMed:11422376, PubMed:1346131). The functional units of these chaperonins consist of heptameric rings of the large subunit Hsp60, which function as a back- to-back double ring. In a cyclic reaction, Hsp60 ring complexes bind one unfolded substrate protein per ring, followed by the binding of ATP and association with 2 heptameric rings of the co-chaperonin Hsp10. This leads to sequestration of the substrate protein in the inner cavity of Hsp60 where, for a certain period of time, it can fold undisturbed by other cell components. Synchronous hydrolysis of ATP in all Hsp60 subunits results in the dissociation of the chaperonin rings and the release of ADP and the folded substrate protein (Probable).
Cellular Location	Mitochondrion matrix.

Background

HSP60 is a member of the chaperonin class of protein factors, which include the Escherichia coli groEL protein and the Rubisco subunit-binding protein of chloroplasts. It acts as a costimulator of human regulatory CD4-positive/CD25 -positive T cells, which inhibit lymphoproliferation and IFNG and TNF secretion by CD4-positive and CD8-positive T cells. HSP60 enhances Treg activity via TLR2, leading to activation of an intracellular signaling cascade that included p38, as well as inhibition of ERK phosphorylation. Suppression of target T cells is mediated by both cell-to-cell contact and by secretion of TGFB and IL10, and it leads to downregulation of ERK, NFKB, and TBET expression. The self-molecule HSP60 can downregulate adaptive immune responses by upregulating Tregs through TLR2 signaling.

Images

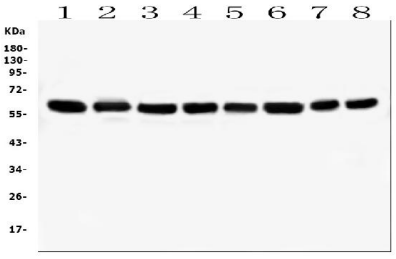


Figure 1. Western blot analysis of HSPD1 using anti-HSPD1 antibody (M01280-3).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours.
The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human Caco-2 whole cell lysates
Lane 2: human A549 whole cell lysates
Lane 3: human THP-1 whole cell lysates
Lane 4: human SW620 whole cell lysates
Lane 5: human U-937 whole cell lysates
Lane 6: human HepG2 whole cell lysates
Lane 7: rat RH35 whole cell lysates
Lane 8: mouse RAW246.7 whole cell lysates
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA 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-HSPD1 antigen affinity purified monoclonal antibody (Catalog # M01280-3) 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 HSPD1 at approximately 60KD. The expected band size for HSPD1 is at 60KD.

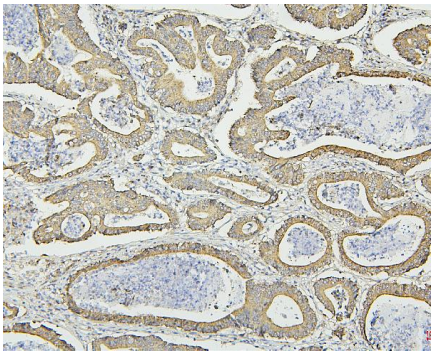


Figure 2. IHC analysis of HSPD1 using anti-HSPD1 antibody (M01280-3).
HSPD1 was detected in paraffin-embedded section of human intestinal cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-HSPD1 Antibody (M01280-3) 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.

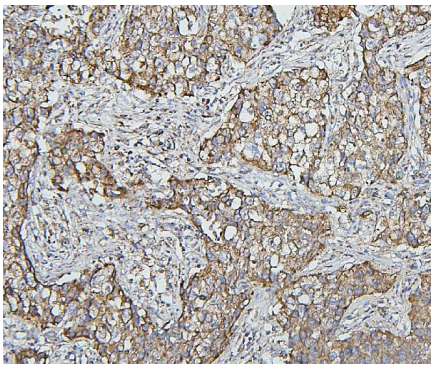


Figure 3. IHC analysis of HSPD1 using anti-HSPD1 antibody (M01280-3).
HSPD1 was detected in paraffin-embedded section of human lung cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-HSPD1 Antibody (M01280-3) 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.

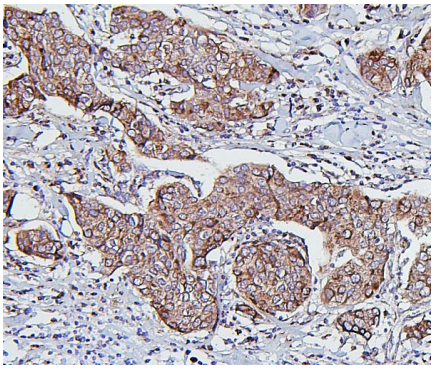
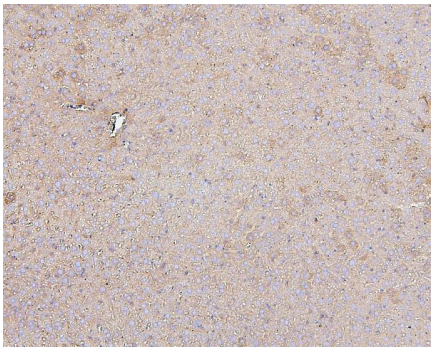


Figure 4. IHC analysis of HSPD1 using anti-HSPD1 antibody (M01280-3).
HSPD1 was detected in paraffin-embedded section of human mammary cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-HSPD1 Antibody (M01280-3) 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 HSPD1 using anti-HSPD1 antibody (M01280-3).
HSPD1 was detected in paraffin-embedded section of mouse liver tissues. Heat mediated antigen retrieval was



performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-HSPD1 Antibody (M01280-3) 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.

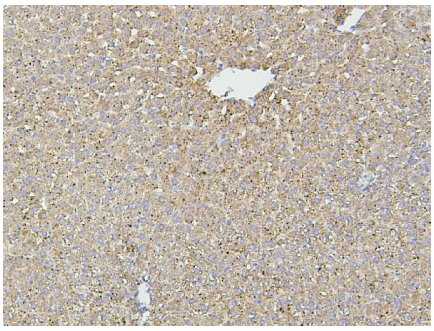


Figure 6. IHC analysis of HSPD1 using anti-HSPD1 antibody (M01280-3).
HSPD1 was detected in paraffin-embedded section of rat liver tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-HSPD1 Antibody (M01280-3) 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.

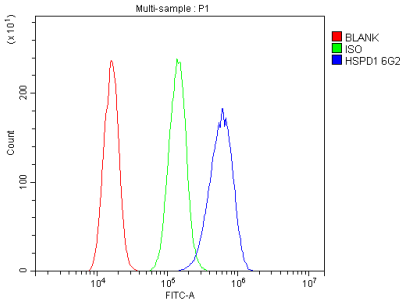


Figure 7. Flow Cytometry analysis of A431 cells using anti-HSPD1 antibody (M01280-3).
Overlay histogram showing A431 cells stained with M01280-3 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-HSPD1 Antibody (M01280-3,1 µg/1x10^6 cells) for 30 min at 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.

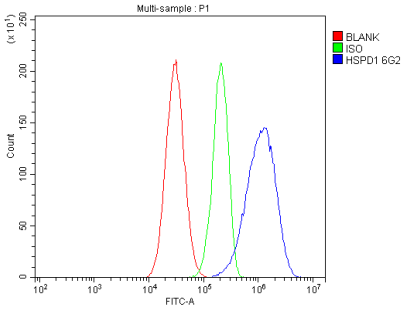
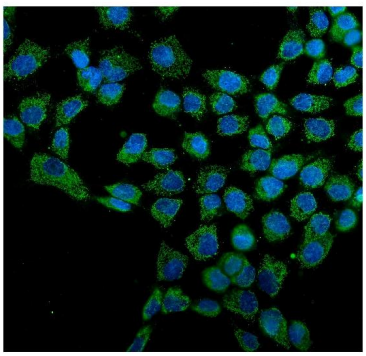


Figure 8. Flow Cytometry analysis of HepG2 cells using anti-HSPD1 antibody (M01280-3).
Overlay histogram showing HepG2 cells stained with M01280-3 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-HSPD1 Antibody (M01280-3,1 µg/1x10^6 cells) for 30 min at 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.

Figure 9. IF analysis of Hsp60/HSPD1 using anti-Hsp60/HSPD1 antibody (M01280-3).
Hsp60/HSPD1 was detected in immunocytochemical section of A431 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-Hsp60/HSPD1 Antibody (M01280-3) 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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