Product datasheet

Anti-HSP60/HSPD1 Antibody (Clone#6G2)

Catalog Number: *M01280-3*

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Basic Information

Product Name	Anti-HSP60/HSPD1 Antibody (Clone#6G2)
Gene Name	HSPD1
Source	Mouse
Clonality	Monoclonal
Isotype	IgG1
Species Reactivity	human, mouse, rat
Tested Application	WB, IHC, FCM, ICC/IF
Contents	500 ug/ml antibody with PBS, 0.02% NaN3, 1 mg/ml BSA and 50% glycerol.
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.
Concentration	500 ug/ml
Purification	protein G purified.
Observed MW	61 kDa
Dilution Ratios	Western blot (WB): 1:500-2000 Immunohistochemistry (IHC): 1:50-400 Immunocytochemistry/Immunofluorescence (ICC/IF): 1:50-400 Flow Cytometry (Fixed): 1:50-200 (Boiling the paraffin sections in 10mM citrate buffer,pH6.0,or PH8.0 EDTA repair liquid for 20 mins is required for the staining of formalin/paraffin sections.) Optimal working dilutions must be determined by end user.

Storage

12 months from date of receipt，-20℃ as supplied. 6 months 2 to 8℃ after reconstitution. Avoid repeated freezing and thawing.

Background Information

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

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TBET expression. The self-molecule HSP60 can downregulate adaptive immune responses by upregulating Tregs through TLR2 signaling.

Reference

Anti-HSP60/HSPD1 Antibody (Clone#6G2)被引用在3文献中。

Selected Validation Data

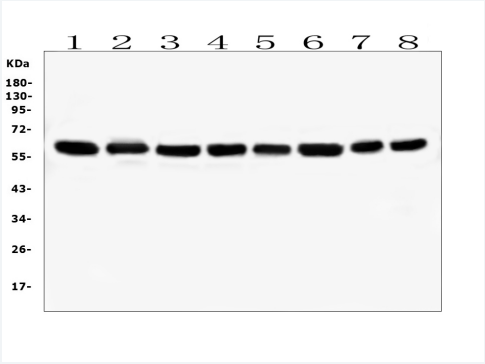

Figure 1. Western blot analysis of HSPD1 using anti-HSPD1 antibody (M01280-3).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

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-

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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.

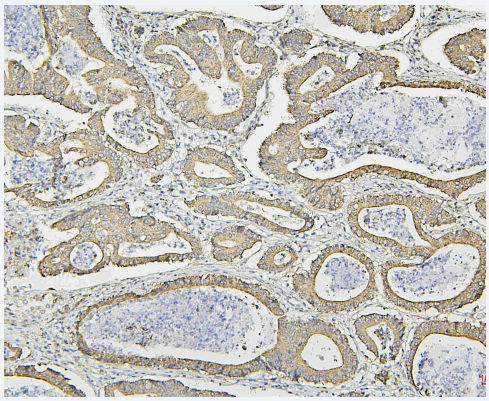

Figure 2. IHC analysis of HSPD1 using anti-HSPD1 antibody (M01280-3).
HSPD1 was detected in paraffin-embedded section of human intestinal cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with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

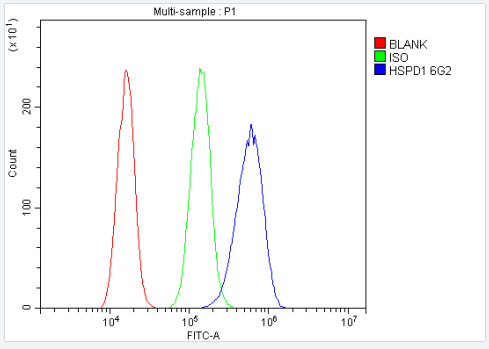

Figure 7. Flow Cytometry analysis of A431 cells using anti-HSP60/HSPD1 antibody (M01280-3).
Overlay histogram showing A431 cells stained with M01280-3 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-HSP60/HSPD1 Antibody (M01280-3) at 1:100 dilution for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG at 1:100 dilution used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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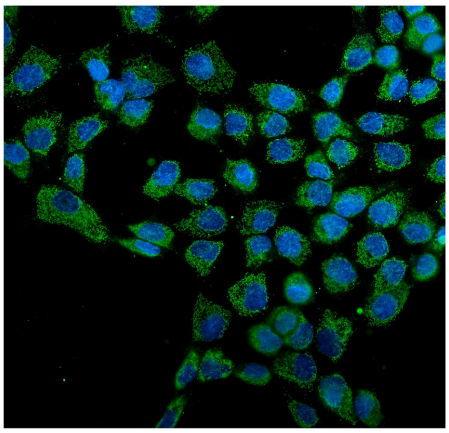

Figure 9. ICC analysis using anti- HSPD1 antibody (M01280-3). was detected in immersion fixed A431 cell line. Cells were stained using the Dylight488-conjugated Anti-mouse IgG Secondary Antibody (green)(Catalog # BA1126) and counterstained with DAPI (blue).