

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

Catalog Number: M01280-3

### About HSPD1

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.

### Overview

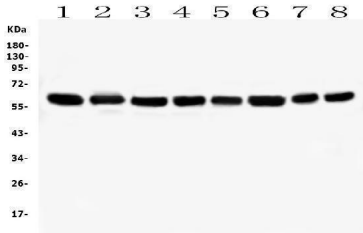
Product Name	Anti-Hsp60/HSPD1 Antibody Picoband® (monoclonal, 6G2)
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-Hsp60/HSPD1 Antibody Picoband® (monoclonal, 6G2) catalog # M01280-3. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Monoclonal 6G2
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na <sub>2</sub> HPO <sub>4</sub> , 0.05mg NaN <sub>3</sub> .
Storage Instructions	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.
Host	Mouse
Uniprot ID	P10809

### Technical Details

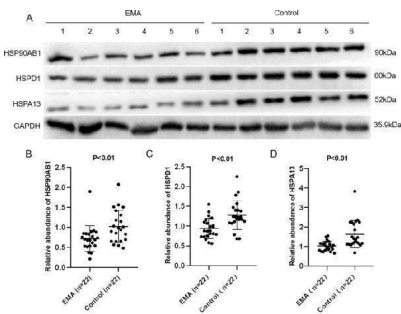
Immunogen	E.coli-derived human Hsp60/HSPD1 recombinant protein (Position: A260-Q496). Human Hsp60 shares 97% amino acid (aa) sequence identity with both mouse and rat Hsp60.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and 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
Concentration	0
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.5ug/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml Immunocytochemistry/Immunofluorescence, 5ug/ml Flow Cytometry (Fixed), 1-3ug/1x10 <sup>6</sup> cells

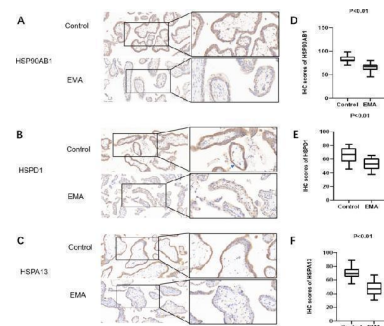
## Anti-Hsp60/HSPD1 Antibody Picoband® (monoclonal, 6G2) (M01280-3) Images



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 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 HSPD1 at approximately 60KD. The expected band size for HSPD1 is at 60KD.

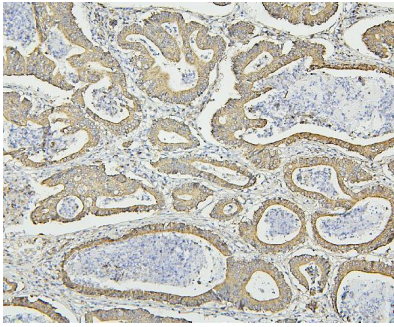


The differential abundance of HSP90AB1, HSPD1 and HSPA13 in villi tissues was verified by Western blot. A The representative of Western blot analysis to verify selected differentially expressed proteins HSP90AB1, HSPD1 and HSPA13 in the villi tissue of embryo from EMA ( n = 6) and control( n = 6). B The scatter plot of HSP90AB1 abundance in the villi tissue of embryo from EMA ( n = 22) vs control ( n = 22) ( P

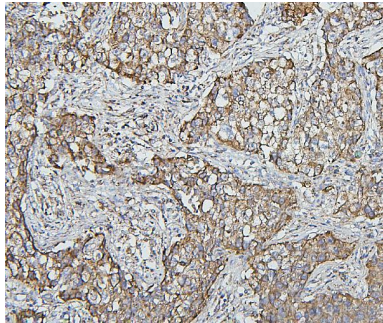


A , B , C Immunohistochemical staining for HSP90AB1, HSPD1, and HSPA13 observed in the cytoplasm of syncytiotrophoblast and cytotrophoblast cells (× 200, × 400). Villus from 22 patients with EMA showed lower; D - F Quantitative scoring results of IHC analyses w shown as box plots Index in PubMed under a CC BY license. PMID: 37587463

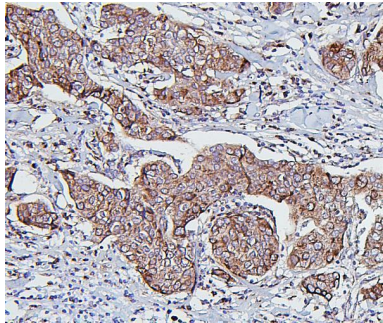
IHC analysis of HSPD1 using anti-HSPD1 antibody (M01280-3). HSPD1 was detected in paraffin-embedded section of human intestinal cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum.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



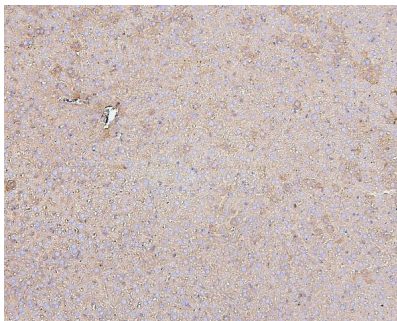
then incubated with 1ug/ml mouse anti-HSPD1 Antibody (M01280-3) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.



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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

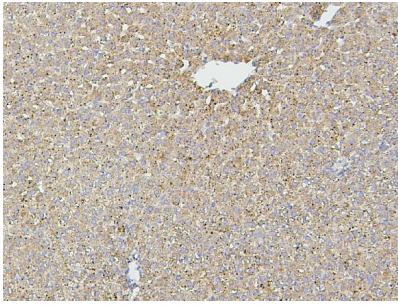


IHC analysis of HSPD1 using anti-HSPD1 antibody (M01280-3). HSPD1 was detected in paraffin-embedded section of human mammary cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

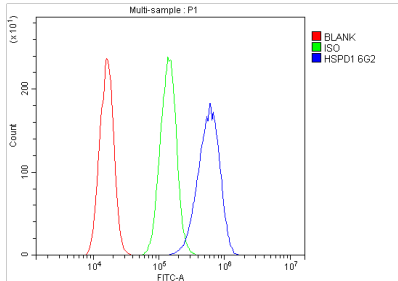


IHC analysis of HSPD1 using anti-HSPD1 antibody (M01280-3). HSPD1 was detected in paraffin-embedded section of mouse liver tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

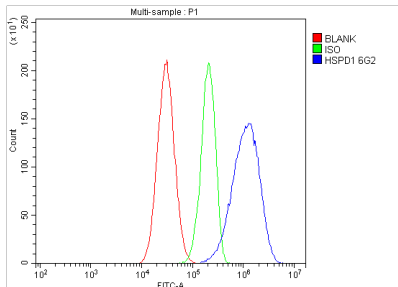
IHC analysis of HSPD1 using anti-HSPD1 antibody (M01280-3). HSPD1 was detected in paraffin-embedded section of rat liver tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with



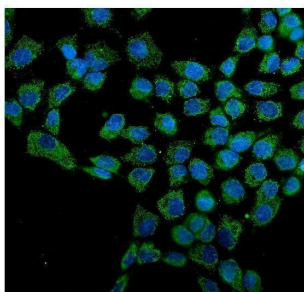
10% goat serum. The tissue section was then incubated with 1ug/ml mouse anti-HSPD1 Antibody (M01280-3) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.



Flow Cytometry analysis of A431 cells using anti-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-HSPD1 Antibody (M01280-3, 1ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



Flow Cytometry analysis of HepG2 cells using anti-HSPD1 antibody (M01280-3). Overlay histogram showing HepG2 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-HSPD1 Antibody (M01280-3, 1ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



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 5ug/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.

1. PubMed ID: 24735884, Hepatic mitochondrial and ER stress induced by defective PPAR $\gamma$  signaling in the pathogenesis of hepatic steatosis
2. PubMed ID: 27698781, Expression and location of HSP60 and HSP10 in the heart tissue of heat-stressed rats
3. PubMed ID: 22500017, Hager L, Li L, Pun H, Liu L, Hossain Ma, Maguire Gf, Naples M, Baker C, Magomedova L, Tam J, Adeli K, Cummins CI, Connelly Pw, Ng Ds. J Biol Chem. 2012 Jun 8;287(24):20755-68. Doi: 10.1074/Jbc.M112.340919. Epub 2012 Apr 12. Lecithin:Cholesterol Ac...

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

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