BOSTER
antibody and ELISA experts

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

Anti-Hsp70/HSPA1A/HSPA1B Antibody

Catalog Number: PA1214

About Hspa1b

The 70 kilodalton heat shock proteins (Hsp70s) are a family of ubiquitously expressed heat shock proteins. The Hsp70s are an important part of the cell's machinery for protein folding, and help to protect cells from stress. All of the Hsp70 proteins have three major functional domains: An N-terminal ATPase domain binds ATP (Adenosine triphosphate) and hydrolyzes it to ADP (Adenosine diphosphate); A substrate binding domain contains a groove with an affinity for neutral, hydrophobic amino acid residues; A C-terminal domain rich in alpha helical structure acts as a 'lid' for the substrate binding domain. By binding tightly to partially-synthesized peptide sequences (incomplete proteins), Hsp70 prevents them from aggregating and being rendered nonfunctional. And it also can act to protect cells from thermal or oxidative stress. Finally, Hsp70 seems to be able to participate in disposal of damaged or defective proteins. Interaction with CHIP (Carboxyl-terminus of Hsp70 Interacting Protein)–an E3 ubiquitin ligase–allows Hsp70 to pass proteins to the cell's ubiquitination and proteolysis pathways.

Overview

Product Name	Anti-Hsp70/HSPA1A/HSPA1B Antibody
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-Hsp70/HSPA1A/HSPA1B Antibody catalog # PA1214. Tested in Flow Cytometry, IF, IHC, IHC-F, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IF, IHC, IHC-F, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg Thimerosal, 0.05mg NaN3.
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	Rabbit
Uniprot ID	P0DMV8/P0DMV9

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human Hsp70, identical to the related rat and mouse sequence.
Predicted Reactive Species	Hamster
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P), IHC(F) and ICC.
Cross Reactivity	No cross-reactivity with other proteins

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

Isotype	Rabbit IgG
Form	Lyophilized
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, 0.1-0.5ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, Mouse, Rat, By Heat Immunohistochemistry (Frozen Section), 0.5-1ug/ml, Mouse, Rat Immunocytochemistry , 0.5-1ug/ml, Human Immunocytochemistry/Immunofluorescence, 2ug/ml, Human Flow Cytometry, 1-3ug/1x10^6 cells, Human

BOSTER
antibody and ELISA experts

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

Anti-Hsp70/HSPA1A/HSPA1B Antibody (PA1214) Images

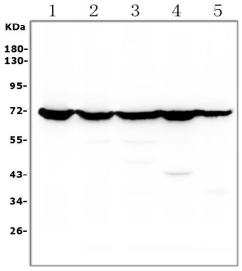


Figure 1. Western blot analysis of Hsp70 using anti-Hsp70 antibody (PA1214).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human HEK293 whole cell lysates,
Lane 2: human Caco-2 whole cell lysates,
Lane 3: human PC-3 whole cell lysates,
Lane 4: human THP-1 whole cell lysates,
Lane 5: human U2OS 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 rabbit anti-Hsp70 antigen affinity purified polyclonal antibody (Catalog # PA1214) 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-rabbit 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 # EK1002) with Tanon 5200 system. A specific band was detected for Hsp70 at approximately 70KD. The expected band size for Hsp70 is at 70KD.

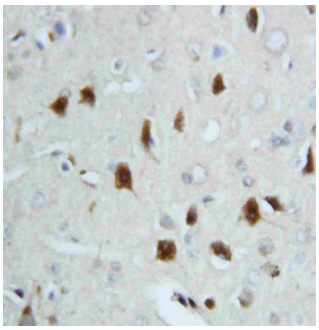


Figure 2. IHC analysis of Hsp70 using anti-Hsp70 antibody (PA1214).
Hsp70 was detected in paraffin-embedded section of rat brain tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-Hsp70 Antibody (PA1214) overnight at 4°C. Biotinylated goat anti-rabbit 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 # SA1022) with DAB as the chromogen.

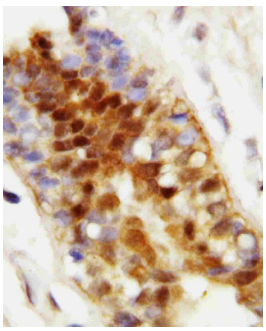
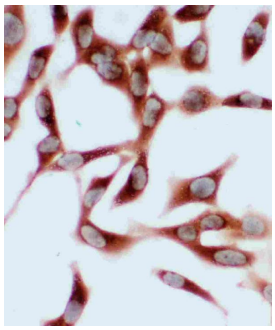


Figure 3. IHC analysis of Hsp70 using anti-Hsp70 antibody (PA1214).
Hsp70 was detected in paraffin-embedded section of mammary cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-Hsp70 Antibody (PA1214) overnight at 4°C. Biotinylated goat anti-rabbit 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 # SA1022) with DAB as the chromogen.

Figure 4. IHC analysis of Hsp70 using anti-Hsp70 antibody

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com



(PA1214).
Hsp70 was detected in immunocytochemical section of HELA cell. 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 1ug/ml rabbit anti-Hsp70 Antibody (PA1214) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

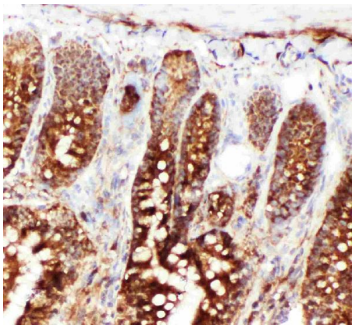


Figure 5. IHC analysis of Hsp70 using anti-Hsp70 antibody (PA1214).
Hsp70 was detected in frozen section of rat intestine tissue. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-Hsp70 Antibody (PA1214) overnight at 4°C. Biotinylated goat anti-rabbit 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 # SA1022) with DAB as the chromogen.

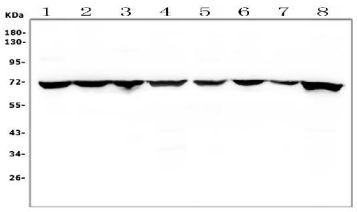
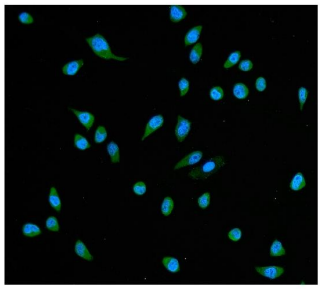


Figure 6. Western blot analysis of Hsp70 using anti-Hsp70 antibody (PA1214).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: rat brain tissue lysates,
Lane 2: rat liver tissue lysates,
Lane 3: rat spleen tissue lysates,
Lane 4: rat PC-12 whole cell lysates,
Lane 5: mouse brain tissue lysates,
Lane 6: mouse liver tissue lysates,
Lane 7: mouse spleen tissue 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 rabbit anti-Hsp70 antigen affinity purified polyclonal antibody (Catalog # PA1214) 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-rabbit 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 # EK1002) with Tanon 5200 system. A specific band was detected for Hsp70 at approximately 70KD. The expected band size for Hsp70 is at 70KD.

Figure 7. IF analysis of Hsp70 using anti-Hsp70 antibody (PA1214).
Hsp70 was detected in immunocytochemical section of Hela 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

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com



incubated with 2ug/mL rabbit anti-Hsp70 Antibody (PA1214) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) 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.

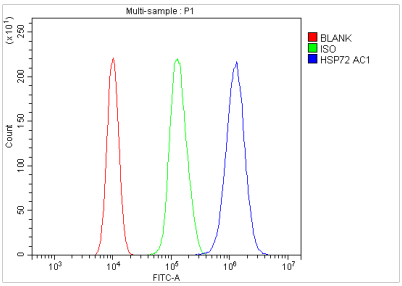


Figure 8. Flow Cytometry analysis of 293T cells using anti-Hsp70 antibody (PA1214).
Overlay histogram showing 293T cells stained with PA1214 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Hsp70 Antibody (PA1214,1ug/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

6 Publications Citing This Product

1. PubMed ID: 31733402, Pan W,Liang J,Tang H,Fang X,Wang F,Ding Y,Huang H,Zhang H.Differentially expressed microRNA profiles in exosomes from vascular smooth muscle cells associated with coronary artery calcification.Int J Biochem Cell Biol.2020 Jan;118:105645.doi:10.1016/j.bioc

2. PubMed ID: 23554704, Effects of minocycline on the expression of NGF and HSP70 and its neuroprotection role following intracerebral hemorrhage in rats

3. PubMed ID: 15378770, Down-modulation of heat shock protein 70 and up-modulation of Caspase-3 during schisandrin B-induced apoptosis in human hepatoma SMMC-7721 cells

Visit bosterbio.com/anti-hsp70-antibody-pa1214-boster.html to see all 6 publications.