

Anti-Hsp70 HSPA1A Antibody Picoband™ (monoclonal, 3H5)

Catalog Number: M00949-2

About HSPA1A

HSPA1 (heat shock 70kDa protein 1A) also known as HSP70-1, HSPA1A, HSP70-1A, HSP72 or HSP70I, is a protein that in humans is encoded by the HSPA1A gene. This intronless gene encodes a 70kDa heat shock protein which is a member of the heat shock protein 70 family. The HSPA1A gene encodes a predicted 641-amino acid protein. The HSPA1 gene is mapped on 6p21.33. Shimizu et al. (1999) found that peripheral blood mononuclear cells of 18 major depression patients expressed a short HSPA1A transcript that utilized exon 1 rather than exon 2, which is found in the more common HSPA1A transcript. No protein was associated with expression of this short HSPA1A mRNA, possibly due to lack of a TATA box or loss of internal ribosome binding sites. Treatment with BGP-15, a pharmacologic inducer of Hsp72 that can protect against obesity-induced insulin resistance, improved muscular architecture, strength, and contractile function in severely affected diaphragm muscles in mdx dystrophic mice.

Overview

Product Name	Anti-Hsp70 HSPA1A Antibody Picoband™ (monoclonal, 3H5)
Reactive Species	Human
Description	Boster Bio Anti-Hsp70 HSPA1A Antibody Picoband™ (monoclonal, 3H5) catalog # M00949-2. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Monoclonal 3H5
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 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	Mouse
Uniprot ID	P0DMV8

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human Hsp70, different from the related mouse sequence by five amino acids, and from the related rat sequence by three amino acids.
Predicted Reactive Species	Hepatitis Virus
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	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	<p>Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.</p> <p>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.</p> <p>Some PubMed article(s) citing the expression level of this target are as follows:</p> <p>Boster Bio's internal QC testing used:</p> <p>Western blot, 0.1-0.5ug/ml</p> <p>Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml</p> <p>Immunocytochemistry/Immunofluorescence, 2ug/ml</p> <p>Flow Cytometry, 1-3ug/1x10⁶ cells</p>

Anti-Hsp70 HSPA1A Antibody Picoband™ (monoclonal, 3H5) (M00949-2) Images

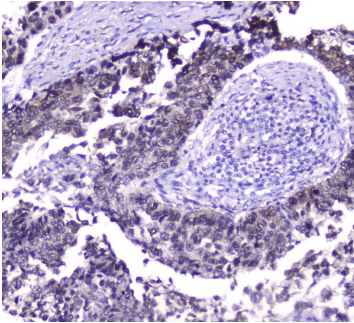


Figure 1. IHC analysis of Hsp70 using anti-Hsp70 antibody (M00949-2).

Hsp70 was detected in paraffin-embedded section of human lung cancer tissue. 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 2ug/ml mouse anti-Hsp70 Antibody (M00949-2) 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.

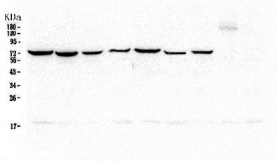


Figure 2. Western blot analysis of Hsp70 using anti-Hsp70 antibody (M00949-2).

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 Hela whole cell lysate,
Lane 2: human COLO-320 whole cell lysate,
Lane 3: human SW620 whole cell lysate,
Lane 4: human A431 whole cell lysate,
Lane 5: human A549 whole cell lysate,
Lane 6: human HepG2 whole cell lysate,
Lane 7: human PANC-1 whole cell lysate.

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-Hsp70 antigen affinity purified monoclonal antibody (Catalog # M00949-2) 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.

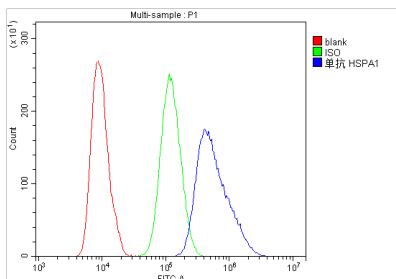


Figure 3. Flow Cytometry analysis of U2OS cells using anti-Hsp70 antibody (M00949-2).

Overlay histogram showing U2OS cells stained with M00949-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Hsp70 Antibody (M00949-2, 1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

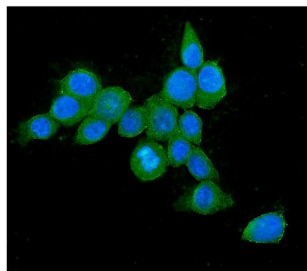


Figure 4. IF analysis of Hsp70 using anti-Hsp70 antibody (M00949-2). Hsp70 was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/mL mouse anti-Hsp70 Antibody (M00949-2) 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.

5 Publications Citing This Product

1. PubMed ID: 23554704, Effects of minocycline on the expression of NGF and HSP70 and its neuroprotection role following intracerebral hemorrhage in rats
2. 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
3. PubMed ID: 22355243, Therapeutic efficacy of trehalose eye drops for treatment of murine dry eye induced by an intelligently controlled environmental system

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