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

Catalog # ABO14779

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P0DMV8
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Hsp70 HSPA1A Antibody Picoband™ (monoclonal, 3H5) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	3303;3304
Other Names	Heat shock 70 kDa protein 1A {ECO:0000312|HGNC:HGNC:5232}, Heat shock 70 kDa protein 1, HSP70-1, HSP70.1, Heat shock protein family A member 1A, HSPA1A, HSP72 {ECO:0000303|PubMed:24318877}, HSPA1, HSX70
Calculated MW	70052
Application Details	Western blot, 0.1-0.5 □g/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml Immunocytochemistry/Immunofluorescence, 2 □g/ml Flow Cytometry, 1-3 □g/1x10^6 cells
Subcellular Localization	Cytoplasm
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Clone Names	Clone: 3H5
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.
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	HSPA1A
Synonyms	HSP72 {ECO:0000303|PubMed:24318877}, HSP
Function	Molecular chaperone implicated in a wide variety of cellular processes, including protection of the proteome from stress, folding and transport of newly synthesized polypeptides, activation of proteolysis of misfolded proteins and the formation and dissociation of protein complexes. Plays a pivotal role in the protein quality control system, ensuring the correct folding of proteins, the re-folding of misfolded proteins and controlling the targeting of proteins for subsequent degradation. This is achieved through cycles of ATP binding, ATP hydrolysis and ADP release, mediated by co-chaperones. The co- chaperones have been shown to not only regulate different steps of the ATPase cycle, but they also have an individual specificity such that one co-chaperone may promote folding of a substrate while another may promote degradation. The affinity for polypeptides is regulated by its nucleotide bound state. In the ATP-bound form, it has a low affinity for substrate proteins. However, upon hydrolysis of the ATP to ADP, it undergoes a conformational change that increases its affinity for substrate proteins. It goes through repeated cycles of ATP hydrolysis and nucleotide exchange, which permits cycles of substrate binding and release. The co-chaperones are of three types: J-domain co-chaperones such as HSP40s (stimulate ATPase hydrolysis by HSP70), the nucleotide exchange factors (NEF) such as BAG1/2/3 (facilitate conversion of HSP70 from the ADP-bound to the ATP-bound state thereby promoting substrate release), and the TPR domain chaperones such as HOPX and STUB1 (PubMed:24012426, PubMed:24318877, PubMed:26865365). Maintains protein homeostasis during cellular stress through two opposing mechanisms: protein refolding and degradation. Its acetylation/deacetylation state determines whether it functions in protein refolding or protein degradation by controlling the competitive binding of co-chaperones HOPX and STUB1. During the early stress response, the acetylated form binds to HOPX which assists in chaperone-mediated protein refolding, thereafter, it is deacetylated and binds to ubiquitin ligase STUB1 that promotes ubiquitin-mediated protein degradation (PubMed:27708256). Regulates centrosome integrity during mitosis, and is required for the maintenance of a functional mitotic centrosome that supports the assembly of a bipolar mitotic spindle (PubMed:27137183). Enhances STUB1-mediated SMAD3 ubiquitination and degradation and facilitates STUB1-mediated inhibition of TGF-beta signaling (PubMed:24613385). Essential for STUB1-mediated ubiquitination and degradation of FOXP3 in regulatory T-cells (Treg) during inflammation (PubMed:23973223). Required as a co-chaperone for optimal STUB1/CHIP ubiquitination of NFATC3 (By similarity). Negatively regulates heat shock-induced HSF1 transcriptional activity during the attenuation and recovery phase period of the heat shock response (PubMed:9499401). Involved in the clearance of misfolded PRDM1/Blimp-1 proteins. Sequesters them in the cytoplasm and promotes their association with SYNV1/HRD1, leading to proteasomal degradation (PubMed:28842558).
Cellular Location	Cytoplasm. Nucleus. Cytoplasm, cytoskeleton, microtubule organizing center, centrosome. Secreted {ECO:0000250|UniProtKB:Q61696}. Note=Localized in cytoplasmic mRNP granules containing untranslated mRNAs

Background

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.

Images

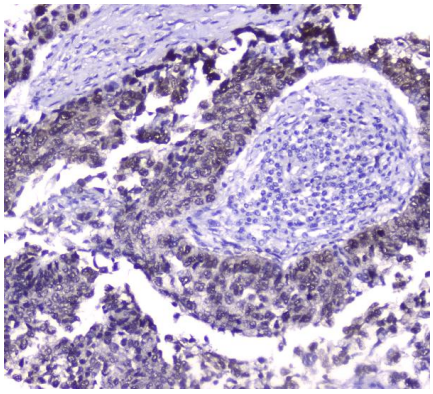


Figure 1. IHC analysis of Hsp70 using anti-Hsp70 antibody (M00949-2).
Hsp70 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

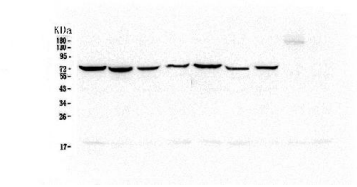
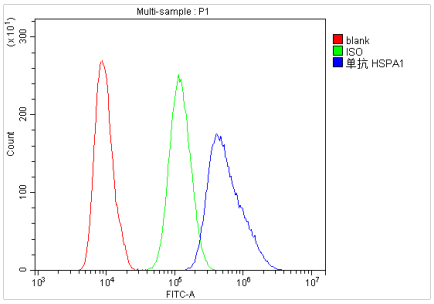


Figure 2. Western blot analysis of Hsp70 using anti-Hsp70 antibody (M00949-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human Hela whole 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 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.

Figure 3. Flow Cytometry analysis of U20S cells using



anti-Hsp70 antibody (M00949-2).
Overlay histogram showing U20S cells stained with M00949-2 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Hsp70 Antibody (M00949-2,1 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.

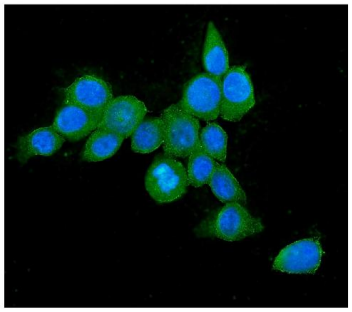


Figure 4. IF analysis of Hsp70 using anti-Hsp70 antibody (M00949-2).
Hsp70 was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with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

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.