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Anti-Hsc70 Antibody Picoband™(monoclonal, 3B6)

Catalog # ABO14915

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P11142
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Hsc70 Antibody Picoband™ (monoclonal, 3B6) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	3312
Other Names	Heat shock cognate 71 kDa protein, 3.6.4.10, Heat shock 70 kDa protein 8, Heat shock protein family A member 8, Lipopolysaccharide-associated protein 1, LAP-1, LPS-associated protein 1, HSPA8 (HGNC:5241)
Calculated MW	70898
Application Details	Western blot, 0.1-0.5 □g/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml, Human, Rat Immunocytochemistry/Immunofluorescence, 2 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6, Human, Mouse
Source	Eukaryota
Protein Name	heat shock protein family A (Hsp70) member 8
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na_2HPO_4, 0.05mg NaN_3.
Clone Names	Clone: 3B6
Immunogen	E.coli-derived human Hsc70 recombinant protein (Position: Q520-A614). Human Hsc70 shares 98.9% amino acid (aa) sequence identity with both mouse and rat Hsc70.
Purification	Immunogen affinity purified.
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	HSPA8 (HGNC:5241)
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, chaperone-mediated autophagy, activation of proteolysis of misfolded proteins, formation and dissociation of protein complexes, and antigen presentation. 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 (PubMed:21148293, PubMed:21150129, PubMed:23018488, PubMed:24732912, PubMed:27916661, PubMed:2799391, PubMed:36586411). This is achieved through cycles of ATP binding, ATP hydrolysis and ADP release, mediated by co-chaperones (PubMed:12526792, PubMed:21148293, PubMed:21150129, PubMed:23018488, PubMed:24732912, PubMed:27916661). The co-chaperones have been shown to not only regulate different steps of the ATPase cycle of HSP70, but they also have an individual specificity such that one co-chaperone may promote folding of a substrate while another may promote degradation (PubMed:12526792, PubMed:21148293, PubMed:21150129, PubMed:23018488, PubMed:24732912, PubMed:27916661). The affinity of HSP70 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. HSP70 goes through repeated cycles of ATP hydrolysis and nucleotide exchange, which permits cycles of substrate binding and release. The HSP70-associated co-chaperones are of three types: J- domain co-chaperones 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:24121476, PubMed:24318877, PubMed:26865365, PubMed:27474739). Plays a critical role in mitochondrial import, delivers preproteins to the mitochondrial import receptor TOMM70 (PubMed:12526792). Acts as a repressor of transcriptional activation. Inhibits the transcriptional coactivator activity of CITED1 on Smad- mediated transcription. Component of the PRP19-CDC5L complex that forms an integral part of the spliceosome and is required for activating pre- mRNA splicing. May have a scaffolding role in the spliceosome assembly as it contacts all other components of the core complex. Binds bacterial lipopolysaccharide (LPS) and mediates LPS-induced inflammatory response, including TNF secretion by monocytes (PubMed:10722728, PubMed:11276205). Substrate recognition component in chaperone-mediated autophagy (CMA), a selective protein degradation process that mediates degradation of proteins with a -KFERQ motif: HSPA8/HSC70 specifically recognizes and binds cytosolic proteins bearing a -KFERQ motif and promotes their recruitment to the surface of the lysosome where they bind to lysosomal protein LAMP2 (PubMed:11559757, PubMed:2799391, PubMed:36586411). KFERQ motif- containing proteins are eventually transported into the lysosomal lumen where they are degraded (PubMed:11559757, PubMed:2799391, PubMed:36586411). In conjunction with LAMP2, facilitates MHC class II presentation of cytoplasmic antigens by guiding antigens to the lysosomal membrane for interaction with LAMP2 which then elicits MHC class II presentation of peptides to the cell membrane (PubMed:15894275). Participates in the ER-associated degradation (ERAD)

quality control pathway in conjunction with J domain-containing co-chaperones and the E3 ligase STUB1 (PubMed:23990462). It is recruited to clathrin-coated vesicles through its interaction with DNAJC6 leading to activation of HSPA8/HSC70 ATPase activity and therefore uncoating of clathrin-coated vesicles (By similarity).

Cellular Location Cytoplasm. Melanosome. Nucleus, nucleolus. Cell membrane. Lysosome membrane; Peripheral membrane protein; Cytoplasmic side. Note=Localized in cytoplasmic mRNP granules containing untranslated mRNAs (PubMed:17289661). Translocates rapidly from the cytoplasm to the nuclei, and especially to the nucleoli, upon heat shock (PubMed:1586970)

Tissue Location Ubiquitous..

Background

HSPA8 (heat shock 70kDa protein 8) also known as HSC70, HSC71, HSP73, HSPA10, FORMERLY, LAP1 or LPS-ASSOCIATED PROTEIN 1, is a heat shock protein that in humans is encoded by the HSPA8 gene. The HSPA8 gene contains 9 exons and spans 5 kb. The deduced HSPA8 protein has 646 amino acids and a predicted molecular mass of 70,899 Da. And the HSPA8 gene is mapped on 11q24.1. HSPA8 plays an important role in cells by transiently associating with nascent polypeptides to facilitate correct folding. HSP73 also functions as an ATPase in the disassembly of clathrin-coated vesicles during transport of membrane components through the cell. Rapid decay involves AU-rich binding protein AUF1, which complexes with heat-shock proteins HSC70 and HSP70, translation initiation factor EIF4G, and poly (A)-binding protein. In the absence of Il3, Hsc70 formed a complex with Hsp40 and Hip, and this complex, in association with Eif4g and Pabp, formed a high-stability complex with Bim mRNA that protected it from ribonucleases.

Images

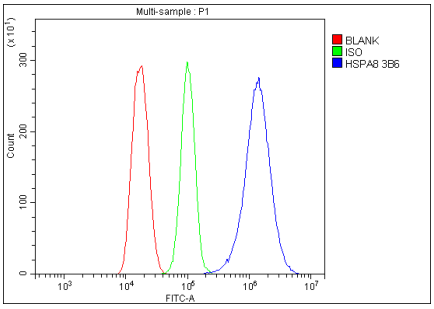


Figure 10. Flow Cytometry analysis of HEPA1-6 cells using anti-Hsc70 antibody (M01024-1).
Overlay histogram showing HEPA1-6 cells stained with M01024-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Hsc70 Antibody (M01024-1, 1 µg/1x10^6 cells) for 30 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.

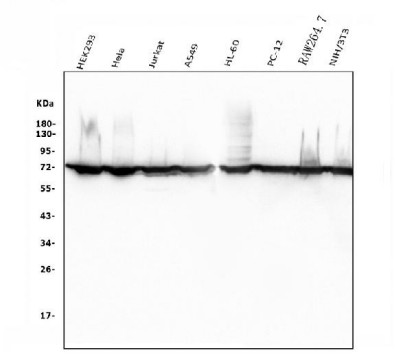


Figure 1. Western blot analysis of Hsc70 using anti-Hsc70 antibody (M01024-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours.
The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human HEK293 whole cell lysates;
Lane 2: human Hela whole cell lysates;
Lane 3: human Jurkat whole cell lysates;
Lane 4: human A549 whole cell lysates;
Lane 5: human HL-60 whole cell lysates;
Lane 6: rat PC-12 whole cell lysates;
Lane 7: human RAW264.7 whole cell lysates;
Lane 8: mouse NIH/3T3 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-Hsc70 antigen affinity purified monoclonal antibody (Catalog # M01024-1) 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. A specific band was detected for Hsc70 at approximately 71KD. The expected band size for Hsc70 is at 71KD.

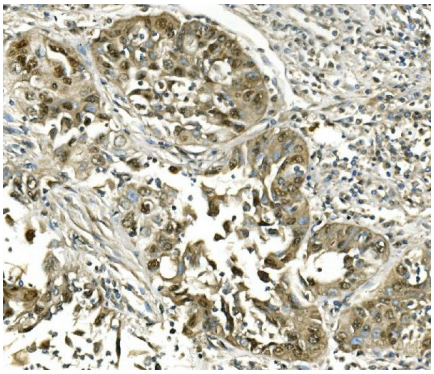


Figure 2. IHC analysis of Hsc70 using anti-Hsc70 antibody (M01024-1).
Hsc70 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Hsc70 Antibody (M01024-1) 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.

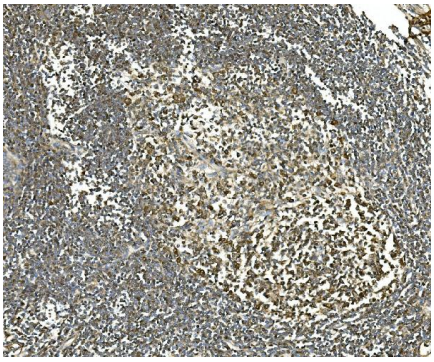


Figure 3. IHC analysis of Hsc70 using anti-Hsc70 antibody (M01024-1).
Hsc70 was detected in paraffin-embedded section of human rectal cancer (lymph node) tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Hsc70 Antibody (M01024-1) 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.

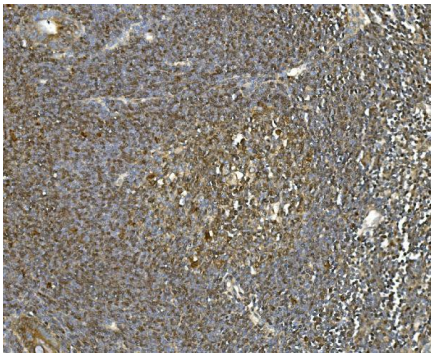
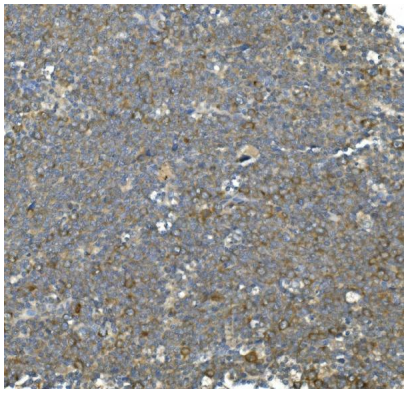


Figure 4. IHC analysis of Hsc70 using anti-Hsc70 antibody (M01024-1).
Hsc70 was detected in paraffin-embedded section of human rectal cancer (lymph node) tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Hsc70 Antibody (M01024-1) 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 5. IHC analysis of Hsc70 using anti-Hsc70 antibody (M01024-1).



Hsc70 was detected in paraffin-embedded section of human tonsil tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Hsc70 Antibody (M01024-1) 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.

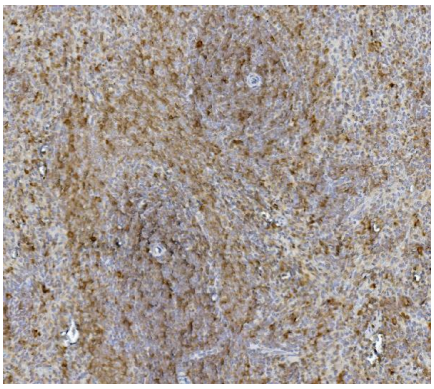


Figure 6. IHC analysis of Hsc70 using anti-Hsc70 antibody (M01024-1).
Hsc70 was detected in paraffin-embedded section of rat spleen tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Hsc70 Antibody (M01024-1) 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.

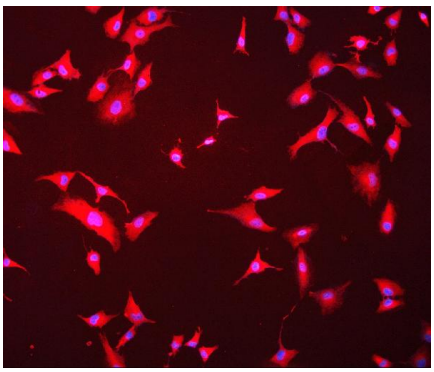


Figure 7. IF analysis of Hsc70 using anti-Hsc70 antibody (M01024-1).
Hsc70 was detected in immunocytochemical section of A549 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-Hsc70 Antibody (M01024-1) overnight at 4°C. DyLight®594 Conjugated Goat Anti-Mouse IgG (BA1141) 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.

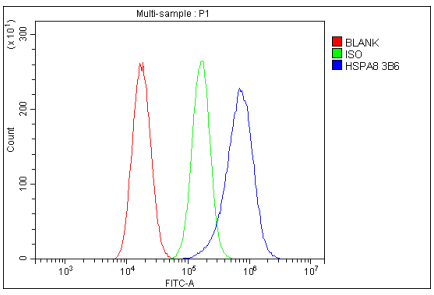
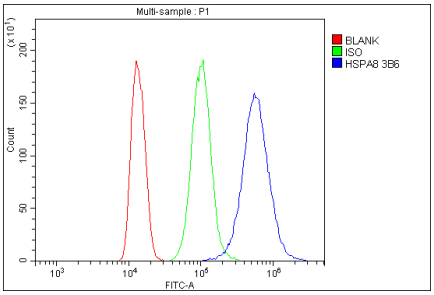


Figure 8. Flow Cytometry analysis of A549 cells using anti-Hsc70 antibody (M01024-1).
Overlay histogram showing A549 cells stained with M01024-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Hsc70 Antibody (M01024-1, 1 µg/1x10^6 cells) for 30 min at 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 9. Flow Cytometry analysis of K562 cells using anti-Hsc70 antibody (M01024-1).
Overlay histogram showing K562 cells stained with M01024-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Hsc70 Antibody (M01024-1, 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.

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