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HSP70 Rabbit pAb

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Catalog # AP94116

Product Information

Application	WB, IHC-P, IHC-F, IF
Primary Accession	P0DMV8
Reactivity	Rat, Human, Mouse
Predicted	Pig, Rabbit, Chicken, Sheep
Host	Rabbit
Clonality	Polyclonal
Calculated MW	70052
Physical State	Liquid
Immunogen	KLH conjugated synthetic peptide derived from human HSP70
Epitope Specificity	500-600/641
Isotype	IgG
Purity	affinity purified by Protein A
Buffer	0.01M TBS (pH7.4) with 1% BSA, 0.02% Proclin300 and 50% Glycerol.
SUBCELLULAR LOCATION	Cytoplasm. Note=Localized in cytoplasmic mRNP granules containing untranslated mRNAs.
Important Note	This product as supplied is intended for research use only, not for use in human, therapeutic or diagnostic applications.
Background Descriptions	This intronless gene encodes a 70kDa heat shock protein which is a member of the heat shock protein 70 family. In conjuction with other heat shock proteins, this protein stabilizes existing proteins against aggregation and mediates the folding of newly translated proteins in the cytosol and in organelles. It is also involved in the ubiquitin-proteasome pathway through interaction with the AU-rich element RNA-binding protein 1. The gene is located in the major histocompatibility complex class III region, in a cluster with two closely related genes which encode similar proteins. [provided by RefSeq, Jul 2008].

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
Dilution	WB=1:1000-5000,IHC-P=1:200-1000,IHC-F=1:200-1000,ICC/IF=1:100-500,IF=1:200-1000,Flow-Cyt=1ug/Test
Storage	Store at -20 °C for one year. Avoid repeated freeze/thaw cycles. When reconstituted in sterile pH 7.4 0.01M PBS or diluent of antibody the antibody is stable for at least two weeks at 2-4 °C.

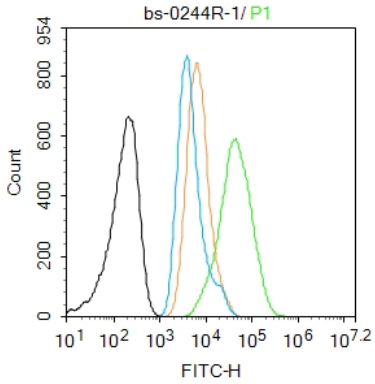
Protein Information

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

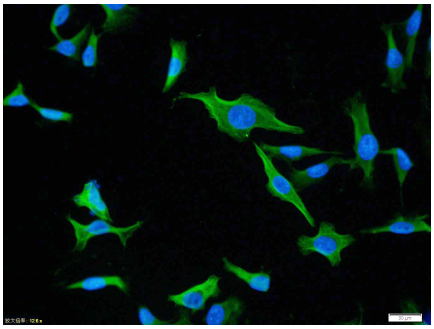
Background

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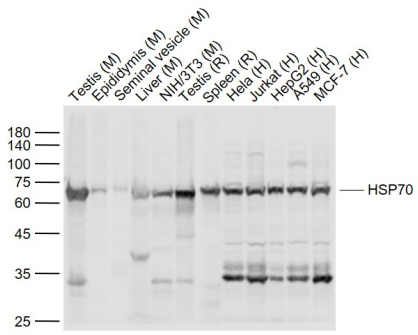
Images



Blank control:A549.
Primary Antibody (green line): Rabbit Anti-HSP70 antibody (AP94116)
Dilution: 1 µg /10^6 cells;
Isotype Control Antibody (orange line): Rabbit IgG .
Secondary Antibody : Goat anti-rabbit IgG-AF488
Dilution: 1 µg /test.
Protocol
The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 90% ice-cold methanol for 20 min at -20°C. The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 30 min at room temperature .Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.

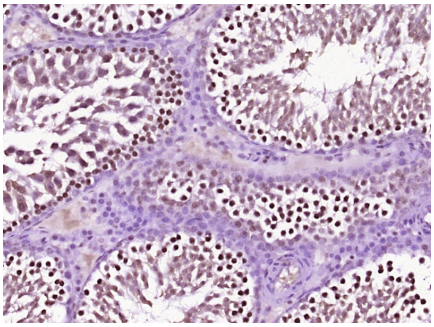


Tissue/cell: U-87MG cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum, C-0005) at 37°C for 20 min; Antibody incubation with (HSP70) polyclonal Antibody, Unconjugated (AP94116) 1:100, 90 minutes at 37°C; followed by a FITC conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue, C02-04002) was used to stain the cell nuclei.



Sample:
Lane 1: Testis (Mouse) Lysate at 40 ug
Lane 2: Epididymis (Mouse) Lysate at 40 ug
Lane 3: Seminal vesicle (Mouse) Lysate at 40 ug
Lane 4: Liver (Mouse) Lysate at 40 ug
Lane 5: NIH/3T3 (Mouse) Cell Lysate at 30 ug
Lane 6: Testis (Rat) Lysate at 40 ug
Lane 7: Spleen (Rat) Lysate at 40 ug
Lane 8: Hela (Human) Cell Lysate at 30 ug
Lane 9: Jurkat (Human) Cell Lysate at 30 ug
Lane 10: HepG2 (Human) Cell Lysate at 30 ug
Lane 11: A549 (Human) Cell Lysate at 30 ug
Lane 12: MCF-7 (Human) Cell Lysate at 30 ug
Primary:
Anti-HSP70 (AP94116) at 1/1000 dilution
Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution
Predicted band size: 70 kD
Observed band size: 68 kD

Paraformaldehyde-fixed, paraffin embedded (Rat testis); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3%



hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (HSP70) Polyclonal Antibody, Unconjugated (AP94116) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.

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