

HSP70 Rabbit pAb

HSP70 Rabbit pAb Catalog # AP93927

Specification

HSP70 Rabbit pAb - Product Information

Application IHC-P, WB
Primary Accession PODMV9
Reactivity Human
Host Rabbit
Clonality Polyclonal
Calculated MW 70052

HSP70 Rabbit pAb - Additional Information

Gene ID 3303;3304

Other Names

Heat shock 70 kDa protein 1B {ECO:0000312|HGNC:HGNC:5233}, Heat shock 70 kDa protein 2, HSP70-2, HSP70.2, Heat shock protein family A member 1B, HSPA1B (HGNC:5233)

Format

0.01M TBS(pH7.4), 0.09% (W/V) sodium azide and 50% Glyce

Storage

Store at -20 $^{\circ}$ 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 $^{\circ}$ C.

HSP70 Rabbit pAb - Protein Information

Name HSPA1B (HGNC:5233)

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).

Cellular Location

Cytoplasm. Cytoplasm, cytoskeleton, microtubule organizing center, centrosome. Note=Localized in cytoplasmic mRNP granules containing untranslated mRNAs

Tissue Location

HSPA1B is testis-specific.

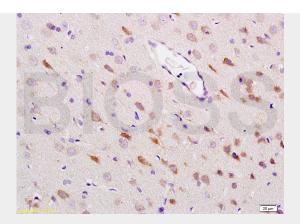
HSP70 Rabbit pAb - Protocols

Provided below are standard protocols that you may find useful for product applications.

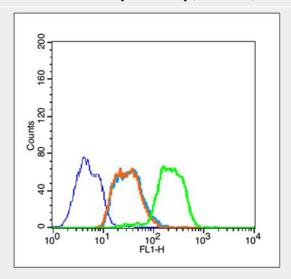
- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- <u>Immunofluorescence</u>
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

HSP70 Rabbit pAb - Images



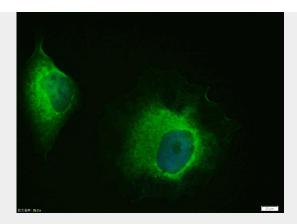


Tissue/cell: rat brain tissue; 4% Paraformaldehyde-fixed and paraffin-embedded; Antigen retrieval: citrate buffer (0.01M, pH 6.0), Boiling bathing for 15min; Block endogenous peroxidase by 3% Hydrogen peroxide for 30min; Blocking buffer (normal goat serum,C-0005) at 37°C for 20 min; Incubation: Anti-HSP-70 Polyclonal Antibody, Unconjugated(AP93927) 1:200, overnight at 4°C, followed by conjugation to the secondary antibody(SP-0023) and DAB(C-0010) staining

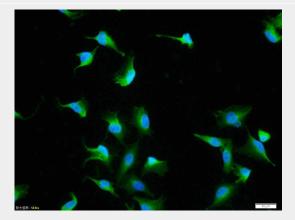


Blank control (blue line): Jurkat (blue). Primary Antibody (green line): Rabbit Anti-HSP70 antibody (AP93927) Dilution: 1 μ g /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG . Secondary Antibody (white blue line): Goat anti-rabbit IgG-FITC Dilution: 1 μ g /test. Protocol The cells were fixed with 2% paraformaldehyde (10 min) , then permeabilized with 90% ice-cold methanol for 30 min on ice. Cells stained with Primary Antibody for 30 min at room temperature. The cells were then incubated in 1 X PBS/2%BSA/10% goat serum to block non-specific protein-protein interactions followed by the antibody for 15 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.

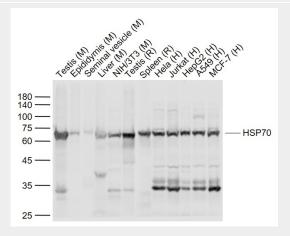




Hela cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum, C-0005) at 37°C for 20 min; Antibody incubation with (HSP70) polyclonal Antibody, Unconjugated (AP93927) 1:100, 90 minutes at 37°C; followed by a conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue, C02-04002) was used to stain the cell nuclei.



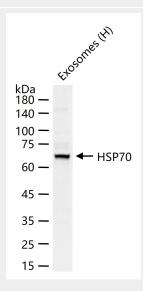
Tissue/cell: A549 cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum, C-0005) at 37°C for 20 min; Antibody incubation with (HSP70) polyclonal Antibody, Unconjugated (AP93927) 1:100, 90 minutes at 37°C; followed by a FITC conjugated Goat Anti-Rabbit 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 (AP93927) at 1/1000 dilution Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution Predicted band size: 70 kD Observed band size: 68 kD



25 ug total protein per lane of various lysates (see on figure) probed with HSP70 polyclonal antibody, unconjugated (AP93927) at 1:1000 dilution and 4°C overnight incubation. Followed by conjugated secondary antibody incubation at r.t. for 60 min.

HSP70 Rabbit pAb - Background

This product as supplied is intended for research use only, not for use in human, therapeutic or diagnostic applications.