

### **HSP70** Rabbit pAb

HSP70 Rabbit pAb Catalog # AP94116

### **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 (<a href="http://www.genenames.org/cgi-bin/gene\_symbol\_report?hgnc\_id=5233" target="blank">HGNC:5233</a>)

#### **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: <a href="http://www.uniprot.org/citations/24012426" target=" blank">24012426</a>, PubMed:<a href="http://www.uniprot.org/citations/24318877" target="blank">24318877</a>, PubMed:<a href="http://www.uniprot.org/citations/26865365" target=" blank">26865365</a>). 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:<a href="http://www.uniprot.org/citations/27708256" target=" blank">27708256</a>). 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: <a href="http://www.uniprot.org/citations/27137183" target=" blank">27137183</a>). Enhances STUB1-mediated SMAD3 ubiquitination and degradation and facilitates STUB1-mediated inhibition of TGF-beta signaling (PubMed:<a href="http://www.uniprot.org/citations/24613385" target=" blank">24613385</a>). Essential for STUB1-mediated ubiquitination and degradation of FOXP3 in regulatory T-cells (Treg) during inflammation (PubMed: <a href="http://www.uniprot.org/citations/23973223" target=" blank">23973223</a>).

#### **Cellular Location**

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

### **Tissue Location**

HSPA1B is testis-specific.

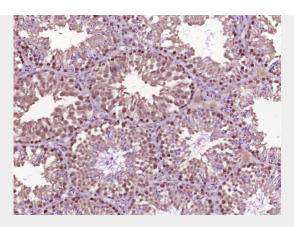
# **HSP70 Rabbit pAb - Protocols**

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

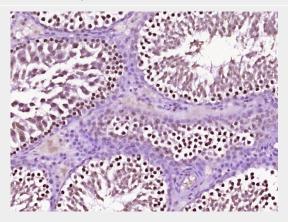
- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- <u>Immunofluorescence</u>
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

### **HSP70 Rabbit pAb - Images**

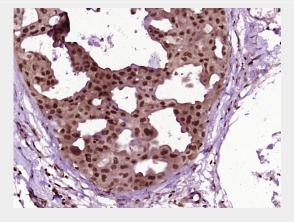




Paraformaldehyde-fixed, paraffin embedded (Mouse testis); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (HSP70) Polyclonal Antibody, Unconjugated (AP94116) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructions and DAB staining.

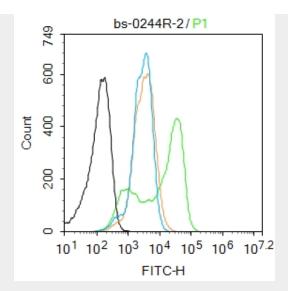


Paraformaldehyde-fixed, paraffin embedded (Rat testis); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (HSP70) Polyclonal Antibody, Unconjugated (AP94116) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructions and DAB staining.

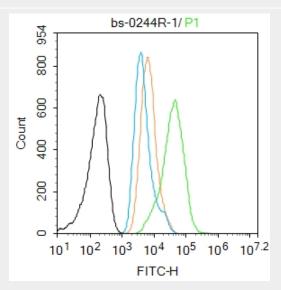


Paraformaldehyde-fixed, paraffin embedded (Human breast carcinoma); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (HSP70) Polyclonal Antibody, Unconjugated (AP94116) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructions and DAB staining.



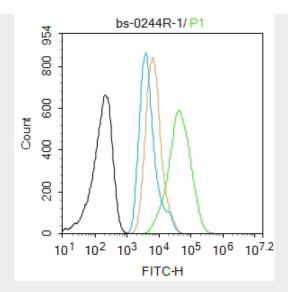


Blank control:293T. Primary Antibody (green line): Rabbit Anti-HSP70 antibody (AP94116) Dilution: 2  $\mu$ g /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG . Secondary Antibody : Goat anti-rabbit IgG-FITC Dilution: 1  $\mu$ g /test. Protocol The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 0.1% PBST for 20 min at room temperature. 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.

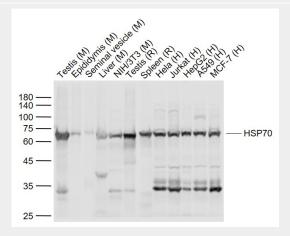


Blank control:A549. Primary Antibody (green line): Rabbit Anti-HSP70 antibody (AP94116) Dilution: 1  $\mu$ g /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG . Secondary Antibody : Goat anti-rabbit IgG-AF488 Dilution: 1  $\mu$ g /test. Protocol The cells were fixed with 4% PFA (10min at 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.





Blank control:A549. Primary Antibody (green line): Rabbit Anti-HSP70 antibody (AP94116) Dilution: 1  $\mu$ g /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG . Secondary Antibody : Goat anti-rabbit IgG-AF488 Dilution: 1  $\mu$ g /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.



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

# **HSP70** Rabbit pAb - Background

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