

Anti-HSP70 Polyclonal Antibody 2

Product Details

Ig Type:	IgG
Reactivity:	Human, Mouse, Rat (predicted: Chicken, Cow, Rabbit, Sheep, Pig)
Molecular Weight:	Theoretical: 70 kDa. Actual: 68 kDa.
Purification:	Protein A purified

Applications

1. Paraformaldehyde-fixed, paraffin embedded (Mouse testis); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (HSP70) Polyclonal Antibody, Unconjugated (TMAB-00894) at 1:400 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.
2. Paraformaldehyde-fixed, paraffin embedded (Rat testis); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (HSP70) Polyclonal Antibody, Unconjugated (TMAB-00894) at 1:400 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.
3. Paraformaldehyde-fixed, paraffin embedded (Human breast carcinoma); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (HSP70) Polyclonal Antibody, Unconjugated (TMAB-00894) at 1:400 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

4. Blank control: 293T.

Primary Antibody (green line): Rabbit Anti-HSP70 antibody (TMAB-00894)

Dilution: 2 µg/10⁶ cells;

Isotype Control Antibody (orange line): Rabbit IgG.

Secondary Antibody: Goat anti-rabbit IgG-FITC

Dilution: 1 µg/test.

Protocol

The cells were fixed with 4% PFA (10 min 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.

5. Blank control: A549. Primary Antibody (green line): Rabbit Anti-HSP70 antibody (TMAB-00894)

Dilution: 1 µg/10⁶ 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 (10 min 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

Verified Activity:

Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature.

6. Blank control: A549. Primary Antibody (green line): Rabbit Anti-HSP70 antibody (TMAB-00894)

Dilution: 1 µg/10⁶ 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 (10 min 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.

7. Sample:

Lane 1: Testis (Mouse) Lysate at 40 µg

Lane 2: Epididymis (Mouse) Lysate at 40 µg

Lane 3: Seminal vesicle (Mouse) Lysate at 40 µg

Lane 4: Liver (Mouse) Lysate at 40 µg

Lane 5: NIH/3T3 (Mouse) Cell Lysate at 30 µg

Lane 6: Testis (Rat) Lysate at 40 µg

Lane 7: Spleen (Rat) Lysate at 40 µg

Lane 8: Hela (Human) Cell Lysate at 30 µg

Lane 9: Jurkat (Human) Cell Lysate at 30 µg

Lane 10: HepG2 (Human) Cell Lysate at 30 µg

Lane 11: A549 (Human) Cell Lysate at 30 µg

Lane 12: MCF-7 (Human) Cell Lysate at 30 µg

Primary:

Anti-HSP70 (TMAB-00894) at 1/1000 dilution

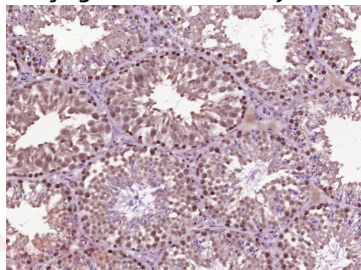
Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution

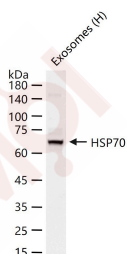
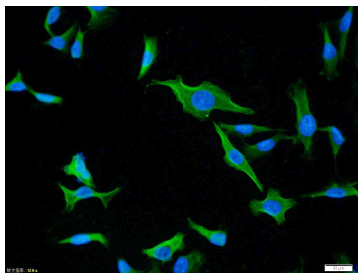
Predicted band size: 70 kDa

Observed band size: 68 kDa

8. Tissue/cell: U-87MG cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum) at 37°C for 20 min; Antibody incubation with (HSP70) polyclonal Antibody, Unconjugated (TMAB-00894) 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) was used to stain the cell nucleus.

9. 25 µg total protein per Lane of various lysates probed with HSP70 polyclonal antibody, unconjugated (TMAB-00894) at 1:1000 dilution and 4°C overnight incubation. Followed by conjugated secondary antibody incubation at RT for 60 min.





Application: FCM,ICC/IF,IF,IHC-Fr,IHC-P,WB

Recommended FCM=1 µg/Test; ICC/IF=1:100-500; IF=1:200-1000; IHC-Fr=1:200-1000; IHC-P=1:200-1000; WB=1:1000-5000

Properties

Stability & Storage: Store at -20°C or -80°C for 12 months. Avoid repeated freeze-thaw cycles.

Shipping: Shipping with blue ice.

Antigen Details

Immunogen: KLH conjugated synthetic peptide: human HSP70

Antigen Species: Human

Gene ID: 3303

Uniprot ID: P0DMV8

Synonyms: heat shock 70kDa protein 1A

Biology Area: Heat Shock Proteins,Tumor biomarkers

Research Background

This intronless gene encodes a 70kDa heat shock protein which is a member of the heat shock protein 70 family. In conjunction 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].

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