Anti-Hsp70 Antibody [SA0379]

ET1601-11

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 70 kDa
Clone number:	SA0379

Description: The 70 kilodalton heat shock proteins (Hsp70s) are a family of conserved ubiquitously expressedheat shock proteins. Proteins with similar structure exist in virtually all living organisms. The Hsp70s are an important part of the cell's machinery for protein folding, and help to protect cells from stress. When not interacting with a substrate peptide, Hsp70 is usually in an ATP bound state. Hsp70 by itself is characterized by a very weak ATPase activity, such that spontaneous hydrolysis will not occur for many minutes. As newly synthesized proteins emerge from the ribosomes, the substrate binding domain of Hsp70 recognizes sequences of hydrophobic amino acid residues, and interacts with them. This spontaneous interaction is reversible, and in the ATP bound state Hsp70 may relatively freely bind and release peptides. However, the presence of a peptide in the binding domain stimulates the ATPase activity of Hsp70, increasing its normally slow rate of ATP hydrolysis.

Immunogen: Recombinant protein within Mouse Hsp70 aa 403-641 / 641.

Positive control: HeLa cell lysate, A549 cell lysate, MCF7 cell lysate, HCT 116 cell lysate, Mouse brain tissue lysate, Mouse testis tissue lysate, Rat testis tissue lysate, NIH/3T3 cell lysate, C2C12 cell lysate, A549, C2C12, NIH/3T3, human breast cancer tissue, human liver cancer tissue, human testis tissue, mouse testis tissue, rat testis tissue, rat prostate tissue, A549.

Subcellular location: Cytoplasm

Database links: SwissProt: P17879 Mouse | Q61696 Mouse | P0DMV9 Human | P0DMV8 Human | Q07439 Rat

Recommended Dilutions:

WB	1:2,000-1:5,000
IF-Cell	1:100
IF-Tissue	1:50
IHC-P	1:500-1:1,500
FC	1:100

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

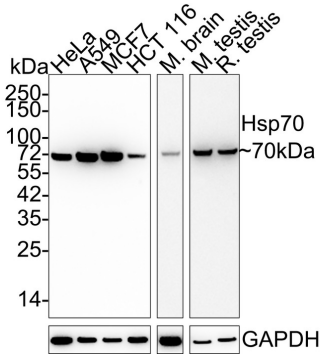

Fig1: Western blot analysis of Hsp70 on different lysates with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: A549 cell lysate
Lane 3: MCF7 cell lysate
Lane 4: HCT 116 cell lysate
Lane 5: Mouse brain tissue lysate
Lane 6: Mouse testis tissue lysate
Lane 7: Rat testis tissue lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 70 kDa
Observed band size: 70 kDa

Exposure time: Lane 1-4: 43 seconds; Lane 5-7: 2 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1601-11) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

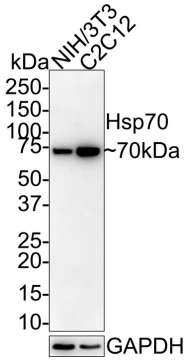

Fig2: Western blot analysis of Hsp70 on different lysates with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/5,000 dilution.

Lane 1: NIH/3T3 cell lysate
Lane 2: C2C12 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 70 kDa
Observed band size: 70 kDa

Exposure time: 5 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1601-11) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

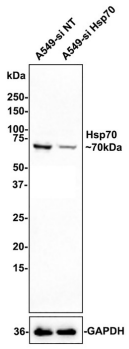

Fig3: Western blot analysis of Hsp70 on different lysates with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/2,000 dilution.

Lane 1: A549-si NT cell lysate
Lane 2: A549-si Hsp70 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 70 kDa
Observed band size: 70 kDa

Exposure time: 49 seconds;
ECL: merk

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1601-11, 1/2,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1/100,000 dilution was used for 1 hour at room temperature.

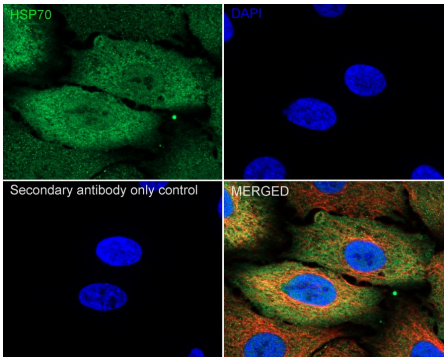

Fig4: Immunocytochemistry analysis of A549 cells labeling Hsp70 with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

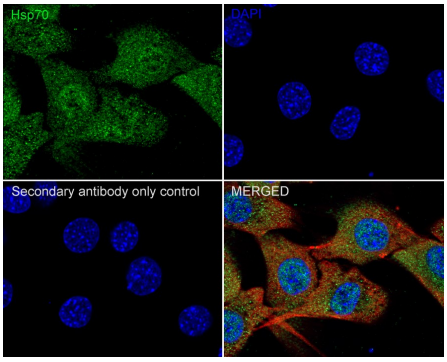

Fig5: Immunocytochemistry analysis of C2C12 cells labeling Hsp70 with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

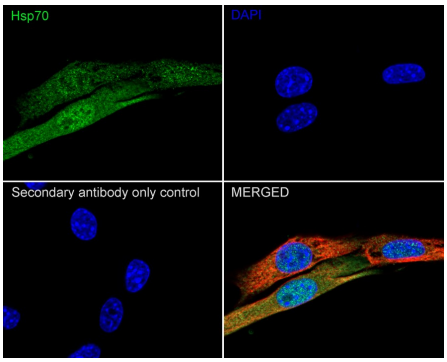

Fig6: Immunocytochemistry analysis of NIH/3T3 cells labeling Hsp70 with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

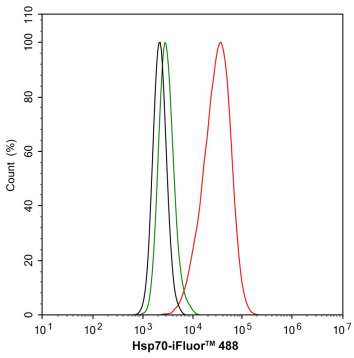

Fig7: Flow cytometric analysis of A549 cells labeling Hsp70.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1601-11, 1/100) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

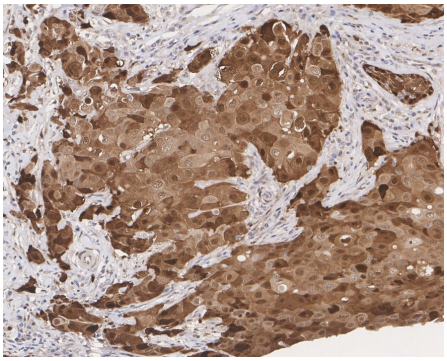

Fig8: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-11) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

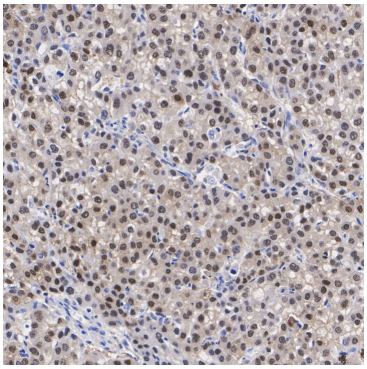

Fig9: Immunohistochemical analysis of paraffin-embedded human liver cancer tissue with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-11) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

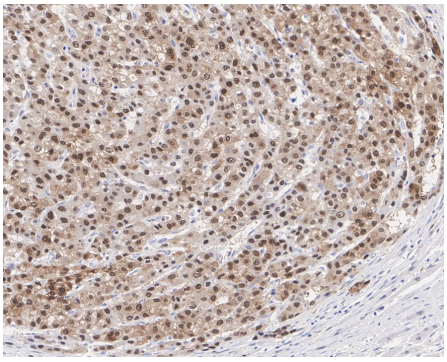

Fig10: Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/1,500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-11) at 1/1,500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

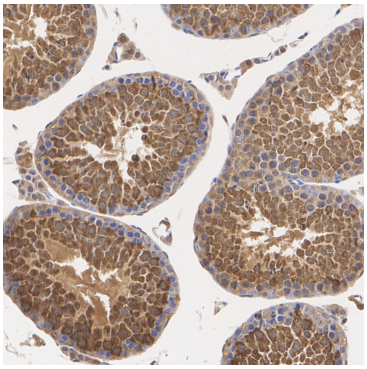

Fig11: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/1,500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-11) at 1/1,500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

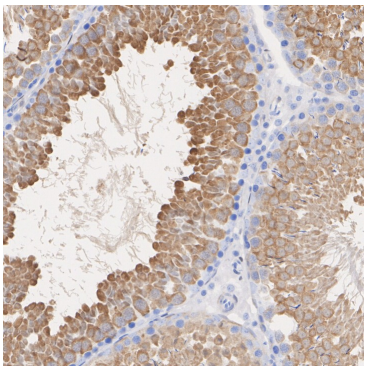

Fig12: Immunohistochemical analysis of paraffin-embedded rat testis tissue with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/1,500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-11) at 1/1,500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

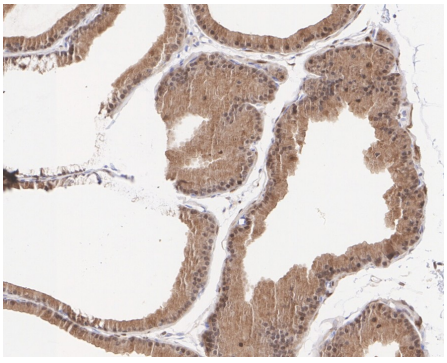

Fig13: Immunohistochemical analysis of paraffin-embedded rat prostate tissue with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-11) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Background References

1. "The disordered amino-terminus of SIMPL interacts with members of the 70-kDa heat-shock protein family." Haag Breese E., Uversky V.N., Georgiadis M.M., Harrington M.A.DNA Cell Biol. 25:704-714(2006)
2. "Hsp70 interacts with the retroviral restriction factor TRIM5alpha and assists the folding of TRIM5alpha." Hwang C.Y., Holl J., Rajan D., Lee Y., Kim S., Um M., Kwon K.S., Song B. J. Biol. Chem. 285:7827-7837(2010)