

Anti-Hsp70 Antibody

ER50802



Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Cow
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 70 kDa

Description: The 70 kilodalton heat shock proteins (Hsp70s) are a family of conserved ubiquitously expressed heat 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: Synthetic peptide within N-terminal human Hsp70.

Positive control: HeLa cell lysate, A549 cell lysate, MCF7 cell lysate, C2C12 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, Rat testis tissue lysate, A549, NIH/3T3, PC-12, human breast cancer tissue, human testis tissue, mouse testis tissue, rat testis tissue.

Subcellular location: Cytoplasm.

Database links: SwissProt: P0DMV8 Human | P0DMV9 Human | P17879 Mouse | Q61696 Mouse | P0DMW0 Rat | P0DMW1 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100-1:250
IHC-P	1:500-1:1,500
FC	1:1,000

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4°C after thawing. Aliquot store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

Purity: Immunogen affinity purified.

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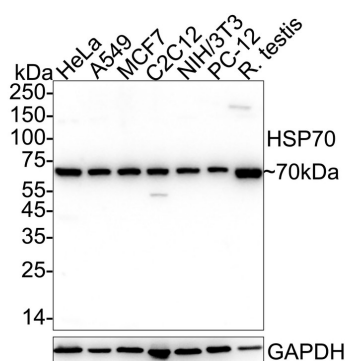
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Fig1: Western blot analysis of Hsp70 on different lysates with Rabbit anti-Hsp70 antibody (ER50802) at 1/2,000 dilution.



Lane 1: HeLa cell lysate (20 µg/Lane)
 Lane 2: A549 cell lysate (20 µg/Lane)
 Lane 3: MCF7 cell lysate (20 µg/Lane)
 Lane 4: C2C12 cell lysate (20 µg/Lane)
 Lane 5: NIH/3T3 cell lysate (20 µg/Lane)
 Lane 6: PC-12 cell lysate (20 µg/Lane)
 Lane 7: Rat testis tissue lysate (40 µg/Lane)

Predicted band size: 70 kDa

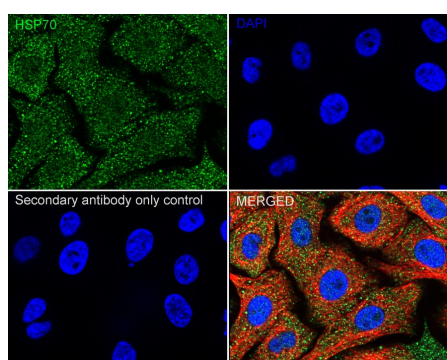
Observed band size: 70 kDa

Exposure time: 4 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (ER50802) at 1/2,000 dilution was used in 5% NFDN/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

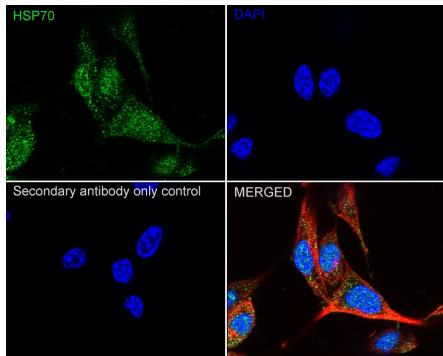
Fig2: Immunocytochemistry analysis of A549 cells labeling Hsp70 with Rabbit anti-Hsp70 antibody (ER50802) at 1/100 dilution.



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 (ER50802) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig3: Immunocytochemistry analysis of NIH/3T3 cells labeling Hsp70 with Rabbit anti-Hsp70 antibody (ER50802) 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 (ER50802) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig4: Immunocytochemistry analysis of PC-12 cells labeling Hsp70 with Rabbit anti-Hsp70 antibody (ER50802) at 1/250 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 (ER50802) at 1/250 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

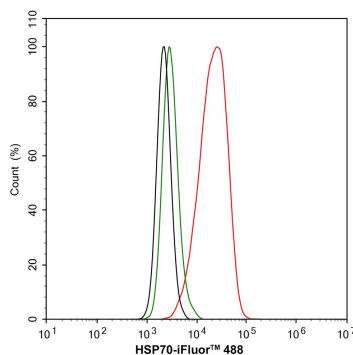


Fig5: Flow cytometric analysis of A549 cells labeling Hsp70.

Cells were fixed and permeabilized. Then stained with the primary antibody (ER50802, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C 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°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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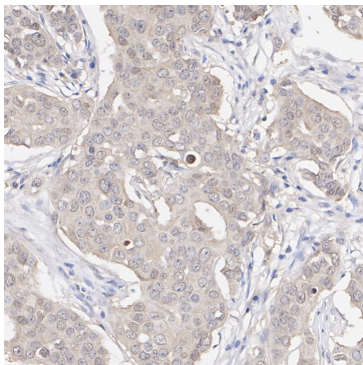


Fig6: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-Hsp70 antibody (ER50802) at 1/500 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ER50802) 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.

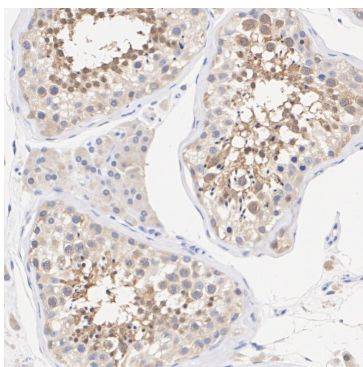


Fig7: Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-Hsp70 antibody (ER50802) at 1/1,500 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ER50802) 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.

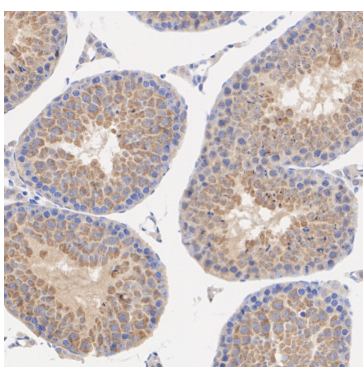


Fig8: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-Hsp70 antibody (ER50802) at 1/1,500 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ER50802) 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.

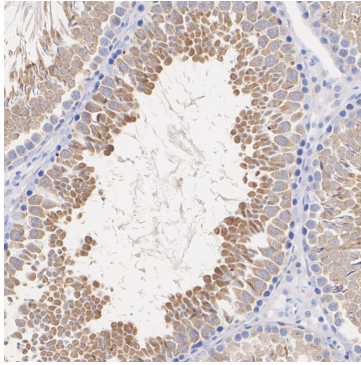


Fig9: Immunohistochemical analysis of paraffin-embedded rat testis tissue with Rabbit anti-Hsp70 antibody (ER50802) at 1/1,500 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ER50802) 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.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. "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)
2. "The molecular chaperone HSP70 binds to and stabilizes NOD2, an important protein involved in Crohn disease." Mohanan V., Grimes C.L. J. Biol. Chem. 289:18987-18998(2014)

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