abcam

Product datasheet

Anti-Hsp70 antibody [3A3] ab5439

Recombinant

*** * * * 3 Abreviews 57 References 9 Images

Overview

Product name Anti-Hsp70 antibody [3A3]

Description Mouse monoclonal [3A3] to Hsp70

Host species Mouse

Specificity This antibody detects several members of the heat shock protein 70 kDa (Hsp 70) gene family

including Hsp 70, Hsc 70, p75, and following heat shock, Hsp 72 from yeast, Drosophila, fish,

mouse, avian, amphibian and human samples.

Tested applications Suitable for: ICC/IF, IHC-P, WB

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Recombinant fragment corresponding to Human Hsp70 aa 504-617 (C terminal).

Epitope Epitope mapping with a panel of Hsp 70 deletion mutants suggests that the epitope recognized is

located between amino acids 504-617 of human Hsp 70, a region that has been shown to be

involved in stress-induced nucleolar localization.

Positive control WB: HeLa, K562, Jurkat, 293T, A431, A549, F9, RAW264.7 and C6, PC-12 lysates. IHC-P:

Human cerebrum and lung carcinoma, Mouse testis, Rat cerebrum tissues. ICC/IF: HeLa and

NIH/3T3, PC-12 cells.

General notes This product has switched from a hybridoma to recombinant production method on 08th March

2021.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.01% Sodium azide

1

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity

Protein A purified

Primary antibody notes

The Hsp 70 family is a set of highly conserved proteins that are induced by a variety of biological stresses, including heat stress, in every organism in which the proteins have been examined. The human Hsp 70 family members include: Hsp 70, a protein which is strongly inducible in all organisms but which is also constitutively expressed in primate cells; Hsp 72, a 72 kDa protein that is induced exclusively under stress conditions; Hsc 70, or cognate protein, is a 72 kDa, constitutively expressed, protein which is involved in the uncoating of clathrin coated vesicles; GRP78, or BiP, is a glucose regulated 78 kDa protein localized in the endoplasmic reticulum; and p75, or Hsp 75, a 75 kDa protein that is found within the mitochondria.

Clonality Monoclonal

Clone number 3A3 Isotype IgG1

Applications

The Abpromise quarantee

Our Abpromise guarantee covers the use of ab5439 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		1/50.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★★★(3)	1/1000. Predicted molecular weight: 70-78 kDa.

Target

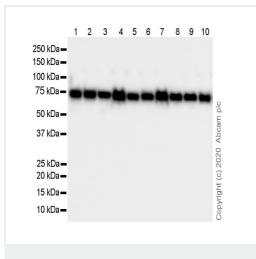
Relevance

Function: In cooperation with other chaperones, the Hsp70 family stabilize preexistent proteins against aggregation and mediate the folding of newly translated polypeptides in the cytosol as well as within organelles. These chaperones participate in all these processes through their ability to recognize nonnative conformations of other proteins. They bind extended peptide segments with a net hydrophobic character exposed by polypeptides during translation and membrane translocation, or following stress-induced damage. In case of rotavirus A infection, serves as a post-attachment receptor for the virus to facilitate entry into the cell. Tissue specificity: HSPA1B is testis-specific.

Cellular localization

Cytoplasm. Localized in cytoplasmic mRNP granules containing untranslated mRNAs.

Images



Western blot - Anti-Hsp70 antibody [3A3] (ab5439)

All lanes: Anti-Hsp70 antibody [3A3] (ab5439) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate

Lane 2: K562 (human chronic myelogenous leukemia lymphoblast), whole cell lysate

Lane 3 : Jurkat (human T cell leukemia T lymphocyte), whole cell lysate

Lane 4 : 293T (human embryonic kidney epithelial cell), whole cell lysate

Lane 5: A431 (human epidermoid carcinoma epithelial cell), whole cell lysate

Lane 6: A549 (human lung carcinoma epithelial cell), whole cell lysate

Lane 7: F9 (mouse embryonal carcinoma epithelial cell), whole cell lysate

Lane 8 : RAW264.7 (mouse Abelson murine leukemia virusinduced tumor macrophage), whole cell lysate

Lane 9: C6 (rat glial tumor glial cell), whole cell lysate

Lane 10: PC-12 (rat adrenal gland pheochromocytoma), whole cell lysate

Lysates/proteins at 10 µg per lane.

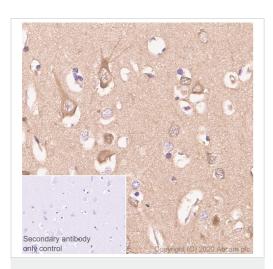
Secondary

All lanes : Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/10000 dilution

Predicted band size: 70-78 kDa **Observed band size:** 70 kDa

Exposure time: 1 second

Blocking/Diluting buffer and concentration: 5% NFDM/TBST

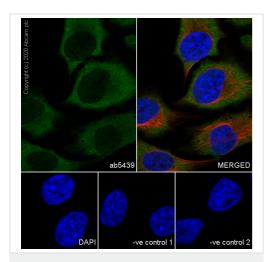


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hsp70 antibody [3A3] (ab5439)

Immunohistochemical analysis of paraffin-embedded Human cerebrum tissue labeling Hsp70 with ab5439 at 1/2000 (0.519 µg/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human cerebrum. The section was incubated with ab5439 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

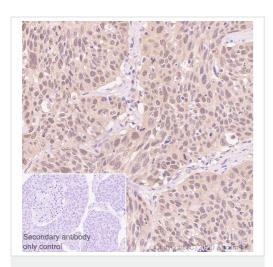
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [3A3] (ab5439)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa cells labelling Hsp70 with ab5439 at 1/50 (20.76 µg/ml) dilution, followed by **ab150113** Goat Anti-Mouse lgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic and weak nuclear staining in HeLa cells **ab179513** Anti-beta Tubulin rabbit monoclonal antibody was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150113</u> Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.

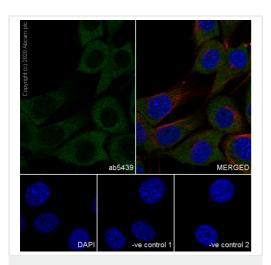


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hsp70 antibody [3A3] (ab5439)

Immunohistochemical analysis of paraffin-embedded Human lung carcinoma tissue labeling Hsp70 with ab5439 at 1/2000 (0.519 µg/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human lung carcinoma. The section was incubated with ab5439 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

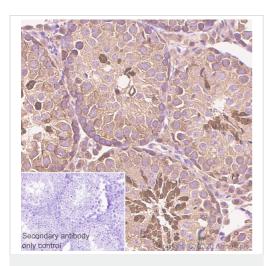
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [3A3] (ab5439)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NIH/3T3 cells labelling Hsp70 with ab5439 at 1/50 (20.76 µg/ml) dilution, followed by **ab150113** Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic and weak nuclear staining in NIH/3T3 cells **ab179513** Anti-beta Tubulin rabbit monoclonal antibody was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150113</u> Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.

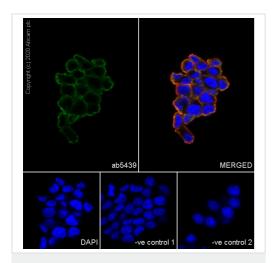


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hsp70 antibody [3A3] (ab5439)

Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labeling Hsp70 with ab5439 at 1/2000 (0.519 μ g/ml) dilution followed by a ready to use LeicaDS9800 (Bond TM Polymer Refine Detection). Positive staining on mouse testis. The section was incubated with ab5439 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

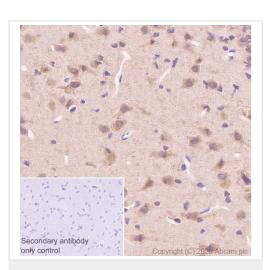
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [3A3] (ab5439)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized PC-12 cells labelling Hsp70 with ab5439 at 1/50 (20.76 µg/ml) dilution, followed by **ab150113** Goat Anti-Mouse lgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in PC-12 cells **ab179513** Anti-beta Tubulin rabbit monoclonal antibody was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150113</u> Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.

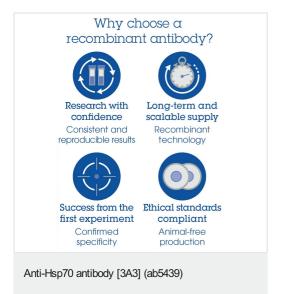


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hsp70 antibody [3A3] (ab5439)

Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labeling Hsp70 with ab5439 at 1/2000 (0.519 μ g/ml) dilution followed by a ready to use LeicaDS9800 (Bond TM Polymer Refine Detection). Positive staining on rat cerebrum. The section was incubated with ab5439 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



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