abcam

ab5442

Anti-Hsp70 antibody [2A4]

Key facts

Isotype	IgM
Host species	Mouse
Storage buffer	Preservative: 0.05% Sodium azide Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Recombinant Fragment Protein within Human HSPA1A. The exact immunogen used to generate this antibody is proprietary information. Database link P0DMV8
Clone number	2A4
Purification technique	Affinity purification Protein A
Specificity	ab5442 detects several members of the heat shock protein 70 kDa (Hsp 70) gene family including Hsp 70, Hsc 70 and, following heat shock, Hsp 72 from yeast, Drosophila, fish, mouse, avian, amphibian and human samples. Immunofluorescence staining of Hsp 70 in heat shocked HeLa cells with ab5442 results in cytoplasmic staining.
Epitope	Epitope mapping with a panel of Hsp 70 deletion mutants suggests that the epitope recognized is located between amino acids 437-479 of human Hsp 70.
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	2 µg/mL
Notes	-

Expected

Species	Mouse
Dilution info	-
Notes	-

Species	Saccharomyces cerevisiae
Dilution info	2 µg/mL
Notes	-

Predicted

Species	Cow, Pig
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	1 µg for 10^6 Cells
Notes	ab91545 - Mouse monoclonal IgM, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse
Dilution info	-
Notes	-

Species	Saccharomyces cerevisiae

Dilution info	1 µg for 10^6 Cells
Notes	ab91545 - Mouse monoclonal IgM, is suitable for use as an isotype control with this antibody.

Predicted

Species	Cow, Pig
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1 µg/mL
Notes	-

Expected

Species	Mouse
Dilution info	-
Notes	-

Species	Saccharomyces cerevisiae
Dilution info	1 µg/mL
Notes	-

Predicted

Species	Cow, Pig
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/200
Notes	Antigen retrieval is not essential but may optimise staining (using a heat mediated method with citrate buffer).

Expected

Species	Mouse
Dilution info	-
Notes	-

Species	Saccharomyces cerevisiae
Dilution info	1/200
Notes	Antigen retrieval is not essential but may optimise staining (using a heat mediated method with citrate buffer).

Predicted

Species	Cow, Pig
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1/100 - 1/200
Notes	-

Species	Human
Dilution info	1/100 - 1/200
Notes	-

Expected

Species	Saccharomyces cerevisiae
Dilution info	1/100 - 1/200
Notes	-

Predicted

Species	Cow, Pig
Dilution info	-
Notes	-

Target data

See full target information HSPA1A

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:24012426, PubMed:24318877, PubMed:26865365). 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:27708256). 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:27137183). Enhances STUB1-mediated SMAD3 ubiquitination and degradation and facilitates STUB1-mediated inhibition of TGF-beta signaling (PubMed:24613385). Essential for STUB1-mediated

ubiquitination and degradation of FOXP3 in regulatory T-cells (Treg) during inflammation (PubMed:23973223). Required as a co-chaperone for optimal STUB1/CHIP ubiquitination of NFATC3 (By similarity). Negatively regulates heat shock-induced HSF1 transcriptional activity during the attenuation and recovery phase period of the heat shock response (PubMed:9499401). Involved in the clearance of misfolded PRDM1/Blimp-1 proteins. Sequesters them in the cytoplasm and promotes their association with SYNV1/HRD1, leading to proteasomal degradation (PubMed:28842558). (Microbial infection) In case of rotavirus A infection, serves as a post-attachment receptor for the virus to facilitate entry into the cell.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Hsp70 also known as Heat Shock Protein 70 or HSPA1B is a molecular chaperone with a mass of approximately 70 kDa. It plays a mechanical role by assisting in the proper folding of nascent polypeptide chains and the refolding of misfolded proteins. Researchers often detect Hsp70 using Western blot and immunohistochemistry (IHC) techniques. Hsp70 is widely expressed in many tissues particularly during stress conditions like heat shock where its expression level increases significantly.
Biological function summary	Hsp70 operates by stabilizing intermediate states of folding proteins preventing aggregation and facilitating the correct folding process. It often forms a complex with co-chaperones such as Hsp40 and nucleotide exchange factors. This complex is essential for the protein's activity and function. Additionally Hsp70 participates in protein degradation pathways by guiding misfolded proteins to the proteasome for degradation maintaining cellular homeostasis.
Pathways	This molecular chaperone plays significant roles in the heat shock response and unfolded protein response pathways. Hsp70 interacts closely with proteins such as

	Hsp90 and co-chaperones which together help protect cells from stress-induced damage. The protein also participates in the JAK/STAT signaling pathway influencing cell proliferation and apoptosis. These interactions suggest an integral role in maintaining cellular integrity during stress conditions.
Associated diseases and disorders	Overexpression of Hsp70 has been associated with various cancers and neurodegenerative diseases. In cancer Hsp70 helps tumor cells survive the hostile tumor microenvironment partly by interacting with anti-apoptotic proteins such as Bcl-2. In neurodegenerative disorders such as Alzheimer's disease Hsp70 associates with amyloid-beta peptides potentially mitigating their aggregation toxicity. These interactions highlight Hsp70's importance in both protective and pathological cellular processes.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

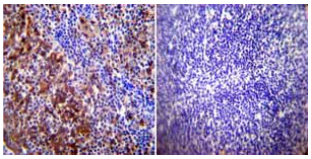
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

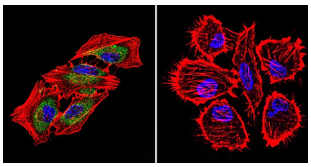
14 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp70 antibody [2A4] (ab5442)

Hsp70 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-Hsp70 antibody

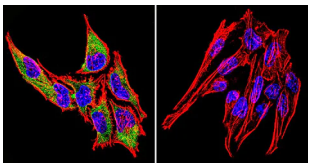
Immunohistochemistry was performed on normal biopsies of deparaffinized Human tonsil tissue. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:200 with a mouse monoclonal antibody recognizing Heat Shock Protein 70 ab5442 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [2A4] (ab5442)

Hsp70 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Hsp70 antibody

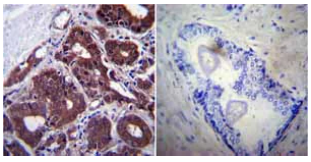
Immunocytochemistry/Immunofluorescence analysis of Hsp70 in NCI-H1299 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with ab5442 at a dilution of 1:100 overnight at 4 C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Hsp70 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [2A4] (ab5442)

Hsp70 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Hsp70 antibody

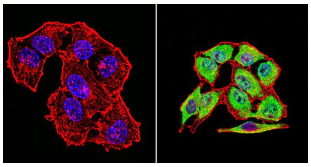
Immunocytochemistry/Immunofluorescence analysis of Hsp70 in HeLa Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were incubated without (control) or with ab5442 at a dilution of 1:200 overnight at 4 C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Hsp70 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp70 antibody [2A4] (ab5442)

Hsp70 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-Hsp70 antibody

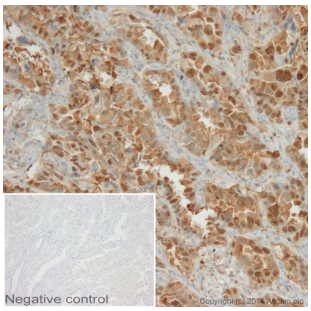
Immunohistochemistry was performed on cancer biopsies of deparaffinized Human prostate carcinoma tissue. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:200 with a mouse monoclonal antibody recognizing Heat Shock Protein 70 ab5442 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [2A4] (ab5442)

Hsp70 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Hsp70 antibody

Immunocytochemistry/Immunofluorescence analysis of Hsp70 (green) in Hela cells. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes at room temperature and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with ab5442 at a dilution of 1:100 and incubated overnight in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody for 45 minutes at room temperature in the dark. F-actin (red) was stained with a fluorescent phalloidin and nuclei (blue) were stained with DAPI. Images were taken at a 60X magnification.

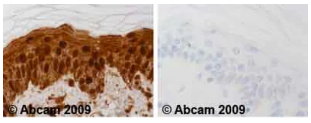


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp70 antibody [2A4] (ab5442)

Hsp70 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-Hsp70 antibody

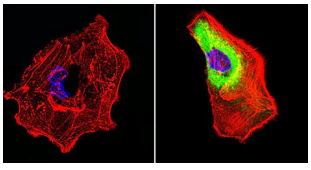
IHC image of Hsp70 staining in human lung formalin fixed paraffin embedded tissue section*. The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH6) for 30mins. The section was incubated with ab5442, 1/2000 dilution overnight at +4°C. An HRP-conjugated secondary (ab97230, 1/2000 dilution) was used for 1hr at room temperature. The section was counterstained with haematoxylin and mounted with DPX.
The inset negative control image is secondary-only at 1/500 dilution.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp70 antibody [2A4] (ab5442)

ab5442 staining human normal skin. Staining is localised to the cytoplasm and nucleus.
Left panel: with primary antibody at 1 ug/ml. Right panel: isotype control.
Sections were stained using an automated system DAKO Autostainer Plus , at room temperature. Sections were rehydrated and antigen retrieved with the Dako 3-in-1 antigen retrieval buffer EDTA pH 9.0 in a DAKO PT Link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 minutes and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required

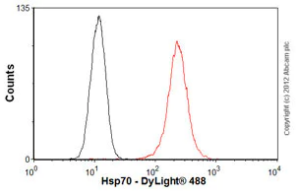


Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [2A4] (ab5442)

Hsp70 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Hsp70 antibody

Immunocytochemistry/Immunofluorescence analysis of Hsp70 (green) in A431 cells. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes at room temperature and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with ab5442 at a dilution of 1:100 and incubated overnight in a humidified chamber. Cells were washed with

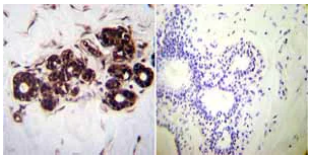
PBST and incubated with a DyLight-conjugated secondary antibody for 45 minutes at room temperature in the dark. F-actin (red) was stained with a fluorescent phalloidin and nuclei (blue) were stained with DAPI. Images were taken at a 60X magnification.



Flow Cytometry - Anti-Hsp70 antibody [2A4] (ab5442)

Hsp70 Flow Cytometry staining using mouse Anti-Hsp70 antibody

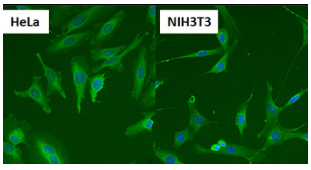
Overlay histogram showing Jurkat cells stained with ab5442 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab5442, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgM (mu chain) (ab97007) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgM [ICIGM] (ab91545, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in Jurkat cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp70 antibody [2A4] (ab5442)

Hsp70 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-Hsp70 antibody

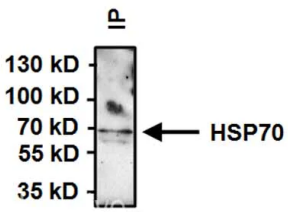
Immunohistochemistry was performed on normal biopsies of deparaffinized Human breast tissue. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:200 with a mouse monoclonal antibody recognizing Heat Shock Protein 70 ab5442 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [2A4] (ab5442)

Hsp70 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Hsp70 antibody

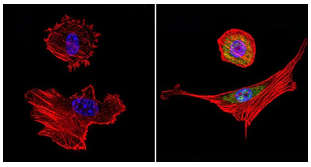
Immunocytochemistry/Immunofluorescence analysis of Hsp70 (green) in HeLa and NIH3T3 cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with ab5442 at a dilution of 1:50 for at least 1 hour at room temperature, washed with PBS, and incubated with fluorescently labeled goat anti-mouse IgM secondary antibody at a dilution of 1:400 for 30 minutes at room temperature. Nuclei (blue) were stained with Hoechst 33342 dye. Images were taken at 20X magnification.



Immunoprecipitation - Anti-Hsp70 antibody [2A4] (ab5442)

Immunoprecipitation of Hsp70 was performed on HeLa cells. Antigen-antibody complexes were formed by incubating 500ug of whole cell lysate with 2ug of HSP70 monoclonal antibody (ab5442) overnight on a rocking platform at 4°C. The immune complexes were captured on 50ul Protein A/G Agarose and eluted with Buffer. Samples were then resolved on a 4-20% Tris-HCl polyacrylamide gel, transferred to a PVDF membraneand blocked with 5% BSA/TBST for at least 1 hour. The membrane was probed with a Hsp70 monoclonal antibody (ab5442) at a dilution of 1:1000 overnight rotating at 4°C then washed in TBST and probed with a goat anti-mouse IgM secondary antibody at a dilution of 1:20000 for at least 1 hour. Chemiluminescent detection was performed.
All lanes:
Immunoprecipitation - Anti-Hsp70 antibody [2A4] (ab5442)

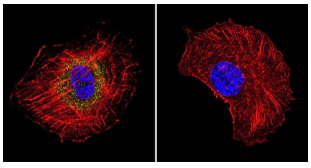
Predicted band size: 70 kDa



Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [2A4] (ab5442)

Hsp70 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Hsp70 antibody

Immunocytochemistry/Immunofluorescence analysis of Hsp70 (green) in NIH-3T3 cells. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes at room temperature and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with ab5442 at a dilution of 1:200 and incubated overnight in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody for 45 minutes at room temperature in the dark. F-actin (red) was stained with a fluorescent phalloidin and nuclei (blue) were stained with DAPI. Images were taken at a 60X magnification.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [2A4] (ab5442)

Hsp70 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Hsp70 antibody

Immunocytochemistry/Immunofluorescence analysis of Hsp70 in NIH-3T3 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with ab5442 at a dilution of 1:100 overnight at 4 C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Hsp70 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.

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