abcam

ab220821

Anti-Hsc70 antibody [EP1531Y] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EP1531Y
Purification technique	Affinity purification Protein A
Concentration	0.949 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information HSPA8

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, chaperone-mediated autophagy, activation of proteolysis of misfolded proteins, formation and dissociation of protein complexes, and antigen presentation. 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 (PubMed:21148293, PubMed:21150129, PubMed:23018488, PubMed:24732912, PubMed:27916661, PubMed:2799391, PubMed:36586411). This is achieved through cycles of ATP binding, ATP hydrolysis and ADP release, mediated by co-chaperones (PubMed:12526792, PubMed:21148293, PubMed:21150129, PubMed:23018488, PubMed:24732912, PubMed:27916661). The co-chaperones have been shown to not only regulate different steps of the ATPase cycle of HSP70, but they also have an individual specificity such that one co-chaperone may promote folding of a substrate while another may promote degradation (PubMed:12526792, PubMed:21148293, PubMed:21150129, PubMed:23018488, PubMed:24732912, PubMed:27916661). The affinity of HSP70 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. HSP70

goes through repeated cycles of ATP hydrolysis and nucleotide exchange, which permits cycles of substrate binding and release. The HSP70-associated co-chaperones are of three types: J-domain co-chaperones 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:24121476, PubMed:24318877, PubMed:26865365, PubMed:27474739). Plays a critical role in mitochondrial import, delivers preproteins to the mitochondrial import receptor TOMM70 (PubMed:12526792). Acts as a repressor of transcriptional activation. Inhibits the transcriptional coactivator activity of CITED1 on Smad-mediated transcription. Component of the PRP19-CDC5L complex that forms an integral part of the spliceosome and is required for activating pre-mRNA splicing. May have a scaffolding role in the spliceosome assembly as it contacts all other components of the core complex. Binds bacterial lipopolysaccharide (LPS) and mediates LPS-induced inflammatory response, including TNF secretion by monocytes (PubMed:10722728, PubMed:11276205). Substrate recognition component in chaperone-mediated autophagy (CMA), a selective protein degradation process that mediates degradation of proteins with a -KFERQ motif: HSPA8/HSC70 specifically recognizes and binds cytosolic proteins bearing a -KFERQ motif and promotes their recruitment to the surface of the lysosome where they bind to lysosomal protein LAMP2 (PubMed:11559757, PubMed:2799391, PubMed:36586411). KFERQ motif-containing proteins are eventually transported into the lysosomal lumen where they are degraded (PubMed:11559757, PubMed:2799391, PubMed:36586411). In conjunction with LAMP2, facilitates MHC class II presentation of cytoplasmic antigens by guiding antigens to the lysosomal membrane for interaction with LAMP2 which then elicits MHC class II presentation of peptides to the cell membrane (PubMed:15894275). Participates in the ER-associated degradation (ERAD) quality control pathway in conjunction with J domain-containing co-chaperones and the E3 ligase STUB1 (PubMed:23990462). It is recruited to clathrin-coated vesicles through its interaction with DNAJC6 leading to activation of HSPA8/HSC70 ATPase activity and therefore uncoating of clathrin-coated vesicles (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab220821 is the carrier-free version of ab51052.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

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

Activity summary	The heat shock cognate 70 (Hsc70) protein also known as HSPA8 plays an important role in chaperone-mediated cellular processes. It exhibits a molecular weight of approximately 70 kDa. Hsc70 is abundantly expressed in various tissues and organs including the brain muscle and liver. As a member of the heat shock protein family Hsc70 maintains protein homeostasis by preventing protein aggregation and assisting in protein folding.
Biological function summary	Hsc70 participates in several processes beyond protein folding. This includes its involvement in the transport of proteins across cellular membranes and the degradation of misfolded proteins. Hsc70 often works in conjunction with co-chaperones and forms part of large protein complexes such as the chaperone machinery involved in clathrin-mediated endocytosis. It also plays a part in cellular stress responses supporting cell survival under various stress conditions.
Pathways	Hsc70 integrates into fundamental cellular pathways such as the ubiquitin-proteasome system and the ER-associated degradation pathway. These pathways are important for protein quality control where Hsc70 collaborates with other heat shock proteins like Hsp40 to facilitate the correct folding and clearance of proteins. In the context of autophagy Hsc70 mediates the recognition and trafficking of specific substrates to lysosomes for degradation demonstrating its importance in maintaining cellular health through these pathways.

Associated diseases and disorders	Hsc70 shows a strong connection to neurodegenerative diseases and cancer. Misregulation of Hsc70 activity can result in protein aggregation seen in disorders such as Parkinson's disease. This situation often links Hsc70 to proteins like alpha-synuclein which accumulate abnormally in these diseases. In cancer altered expression of Hsc70 can promote tumor development by hyper-accommodating the stress conditions within the tumor microenvironment. Hsc70's interplay with proteins such as p53 highlights its role in the pathology of tumorigenesis.

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

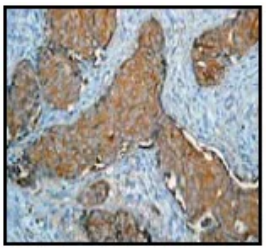
We do not recommend this combination. It is not covered by our product promise.

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.

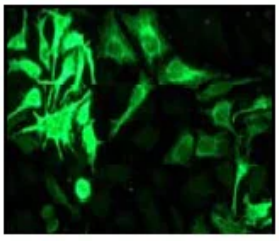
8 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsc70 antibody [EP1531Y] - BSA and Azide free (ab220821)

Hsc70 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Hsc70 antibody

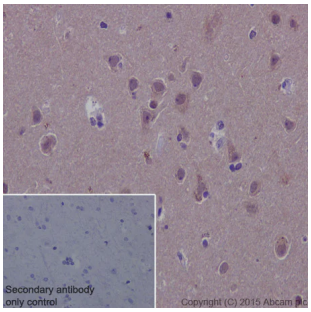
Unpurified ab51052 (1/250) staining Hsc70 in paraffin embedded human breast carcinoma tissue by Immunohistochemistry.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab51052).



Immunocytochemistry/ Immunofluorescence - Anti-Hsc70 antibody [EP1531Y] - BSA and Azide free (ab220821)

Hsc70 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Hsc70 antibody

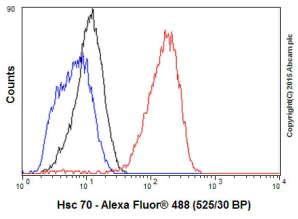
Immunofluorescent staining of HeLa cells using unpurified ab51052 (1/100).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab51052).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsc70 antibody [EP1531Y] - BSA and Azide free (ab220821)

Hsc70 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Hsc70 antibody

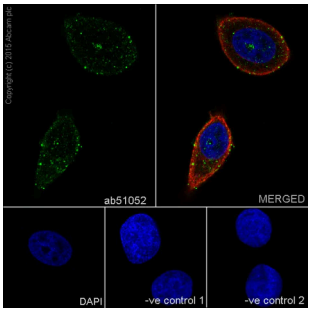
Immunohistochemical staining of paraffin embedded human cerebral cortex with purified ab51052 at a working dilution of 1/500. The secondary antibody used is HRP goat anti-rabbit IgG H&L (ab97051) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab51052).



Flow Cytometry (Intracellular) - Anti-Hsc70 antibody [EP1531Y] - BSA and Azide free (ab220821)

Hsc70 Flow Cytometry (Intracellular) staining using rabbit Anti-Hsc70 antibody

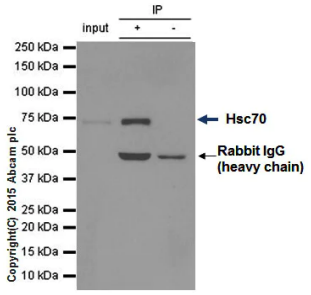
Overlay histogram showing MCF7 cells fixed in 4% PFA and stained with purified ab51052 at a dilution of 1 in 70 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab51052).



Immunocytochemistry/ Immunofluorescence - Anti-Hsc70 antibody [EP1531Y] - BSA and Azide free (ab220821)

Hsc70 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Hsc70 antibody

Immunofluorescence staining of MCF7 cells with purified ab51052 at a working dilution of 1/250, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab51052 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab51052).

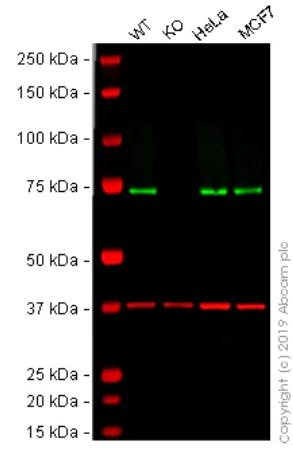


Immunoprecipitation - Anti-Hsc70 antibody [EP1531Y] - BSA and Azide free (ab220821)

ab51052 (purified) at 1/20 immunoprecipitating Hsc70 in 10 µg HeLa (Lanes 1 and 2, observed at 71 kDa). Lane 3 - PBS. For western blotting, HRP Veriblot for IP (ab131366) was used for detection at 1/1000 dilution. Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab51052).

All lanes:
Immunoprecipitation - Anti-Hsc70 antibody [EP1531Y] (ab51052)

Predicted band size: 70 kDa



Western blot - Anti-Hsc70 antibody [EP1531Y] - BSA and Azide free (ab220821)

This data was developed using the same antibody clone in a different buffer formulation (ab51052).
Lanes 1 - 4: Merged signal (red and green). Green - ab51052 observed at 73 kDa. Red - loading control, ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab51052 was shown to react with HSPA8 in A431 wild-type cells in Western blot. Loss of signal was observed when HSPA8 knockout sample was used. A431 wild-type and HSPA8 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% Milk in TBS-T (0.1% Tween®) before incubation with ab51052 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 500 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Hsc70 antibody [EP1531Y] - BSA and Azide free (ab220821) at 1/500 dilution

Lane 1:
Wild-type A431 whole cell lysate at 20 µg

Lane 2:
HSPA8 knockout A431 whole cell lysate at 20 µg

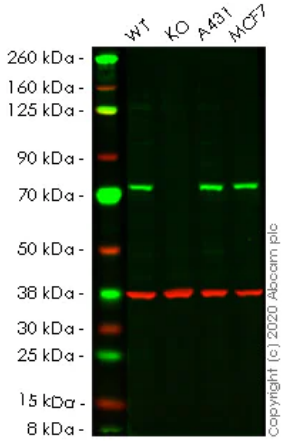
Lane 3:
HeLa whole cell lysate at 20 µg

Lane 4:
MCF7 whole cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 70 kDa

Observed band size: 73 kDa



Western blot - Anti-Hsc70 antibody [EP1531Y] - BSA and Azide free (ab220821)

Hsc70 Western blot staining using rabbit Anti-Hsc70 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab51052).
Lanes 1- 4: Merged signal (red and green). Green - ab51052 observed at 71 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab51052 was shown to react with Hsc70 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265664 (knockout cell lysate ab256944) was used. Wild-type HeLa and HSPA8 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab51052 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 500 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Hsc70 antibody [EP1531Y] (ab51052) at 1/500 dilution

Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human HSPA8 (Hsc70) knockout HeLa cell lysate (ab256944) at 20 µg

Lane 3:
A431 cell lysate at 20 µg

Lane 4:
MCF7 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 70 kDa

Observed band size: 71 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.