Anti-Hsp70 [2A4] antibody ab5442

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Overview

Product name	Anti-Hsp70 [2A4] antibody
Description	Mouse monoclonal [2A4] to Hsp70
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.
Tested applications	Flow Cyt, IP, ICC/IF, IHC-P, WB
Species reactivity	**Reacts with:** Mouse, Rat, Chicken, Human, Saccharomyces cerevisiae, Fruit fly (Drosophila melanogaster), Fish, Amphibians **Predicted to work with:** Cow, Pig, Non Human Primates
Immunogen	Recombinant fragment corresponding to Human Hsp70.
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.
Positive control	ICC: heat shocked HeLa cells

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.05% Sodium azide Constituent: 99% PBS
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	2A4
Isotype	IgM
Research Areas	Signal Transduction → Protein Trafficking → Chaperones → Heat Shock Proteins Cancer → Tumor biomarkers → Other

Applications

Our Abpromise guarantee covers the use of **ab5442** in the following tested applications.

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

Application	Notes
Flow Cyt	Flow Cyt: Use 1µg for 10^6 cells.
IP	IP: Use a concentration of 2 µg/ml.
ICC/IF	ICC/IF: 1/100 - 1/200.

IHC-P	IHC-P: 1/200. Antigen retrieval is not essential but may optimise staining (using a heat mediated method with citrate buffer).
WB	WB: 1/1000 - 1/2500. Detects a band of approximately 70-72 kDa representing different members of the Hsp 70 family. 2-dimensional gel electrophoresis is required to resolve the heat induced form of these proteins from their constitutively expressed counterparts.

Target

Function	In cooperation with other chaperones, Hsp70s 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.
Sequence similarities	Belongs to the heat shock protein 70 family.
Cellular localization	Cytoplasm. Localized in cytoplasmic mRNP granules containing untranslated mRNAs.

Target information above from: UniProt accession P08107 The UniProt Consortium

The Universal Protein Resource (UniProt) in 2010

Nucleic Acids Res. 38:D142-D148 (2010) .

Database links

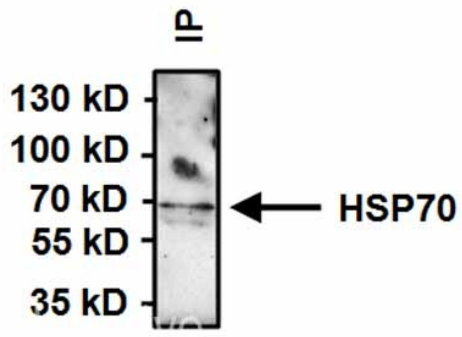
Entrez Gene: 423504 Chicken
Entrez Gene: 281825 Cow
Entrez Gene: 3303 Human
Entrez Gene: 3304 Human
Entrez Gene: 15511 Mouse
Entrez Gene: 193740 Mouse
Entrez Gene: 396906 Pig
Entrez Gene: 24472 Rat
Entrez Gene: 294254 Rat
Omim: 140550 Human
SwissProt: Q27975 Cow
SwissProt: P08107 Human
SwissProt: P17879 Mouse
SwissProt: Q61696 Mouse
SwissProt: P34930 Pig
SwissProt: Q07439 Rat
Unigene: 274402 Human
Unigene: 719966 Human
Unigene: 728810 Human
Unigene: 1950 Rat
Unigene: 228225 Rat

Alternative names

DAQB 147D11.1 001 antibody
FLJ54303 antibody
FLJ54370 antibody
FLJ54392 antibody
FLJ54408 antibody
FLJ75127 antibody
Heat shock 70 kDa protein 1 antibody

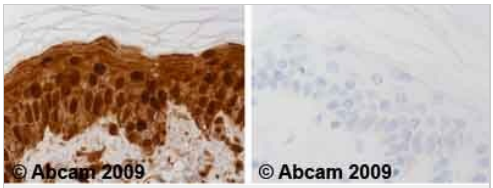
Heat shock 70 kDa protein 1/2 antibody
Heat shock 70 kDa protein 1A/1B antibody
heat shock 70kDa protein 1A antibody
Heat shock 70kDa protein 1B antibody
Heat shock induced protein antibody
heat shock protein 70 antibody
HSP70 1 antibody
HSP70 2 antibody
HSP70-1/HSP70-2 antibody
HSP70-1A antibody
HSP70.1 antibody
HSP70.1/HSP70.2 antibody
HSP70I antibody
HSP71_HUMAN antibody
HSP72 antibody
HSPA1 antibody
HSPA1A antibody
HSPA1B antibody
XXbac BCX40G17.3 001 antibody

Anti-Hsp70 [2A4] antibody images



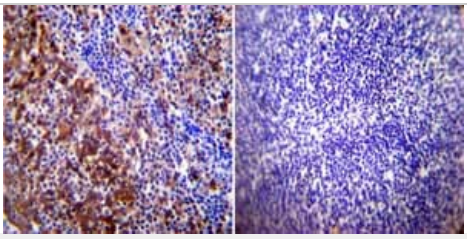
Immunoprecipitation - Anti-Hsp70 antibody [2A4] (ab5442)

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.



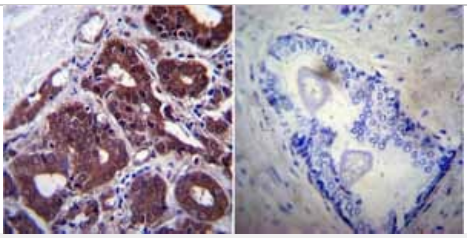
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections)-Hsp70 antibody [2A4](ab5442)

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



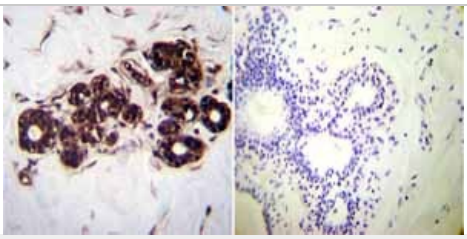
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections)-Anti-Hsp70 antibody [2A4](ab5442)

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.



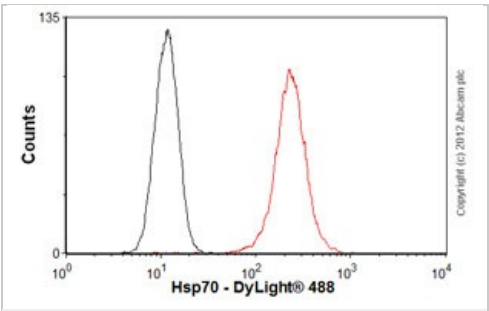
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections)-Anti-Hsp70 antibody [2A4](ab5442)

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.



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

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.



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

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 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgM (mu chain) (ab97007) at 1/500 dilution for 30 min at 22ºC. 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.

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