abcam

Product datasheet

Anti-Hsp70 antibody [N27F3-4] ab47454

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Product name	Anti-Hsp70 antibody [N27F3-4]	
Description	Mouse monoclonal [N27F3-4] to Hsp70	
Host species	Mouse	
Specificity	Detects 72 and 73kDa proteins corresponding to the molecular mass of inducible Hsp and Hsc70 of SDS PAGE immunoblots.	
Tested applications	Suitable for: IHC-P, WB, IP, ICC/IF	
Species reactivity	ies reactivity Reacts with: Mouse, Rat, Human	
	Predicted to work with: Plants	
Immunogen	Recombinant full length protein corresponding to Human Hsp70. Recombinant hsp70/hsc70 (Human) Database link: <u>P11142, P34932</u>	
Positive control	HeLa nuclear extract lysate (ab14655) can be used as a positive control in WB. ICC/IF: Hek293 cells.	
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.	
	If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As	

Properties	
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Form	Liquid	
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.	
Storage buffer	Preservative: 0.09% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine)	
Purity	Protein G purified	
Clonality	Monoclonal	
Clone number	N27F3-4	

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab47454 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
IHC-P		1/1000.
WB	$\star \star \star \star \star (2)$	1/1000. Predicted molecular weight: 70 kDa.
IP		Use a concentration of 5 µg/ml.
ICC/IF		1/50.

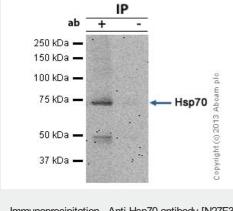
Target

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

Immunoprecipitation - Anti-Hsp70 antibody [N27F3-4] (ab47454) Hsp70 was immunoprecipitated using 0.5mg Rat Testis tissue lysate, 5µg of Mouse monoclonal to Hsp70 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

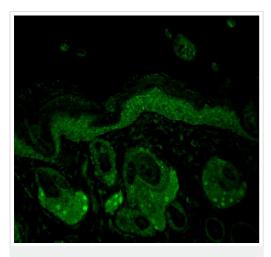
The antibody was incubated under agitation with Protein G beads for 10min, Rat Testis tissue lysate lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab47454.

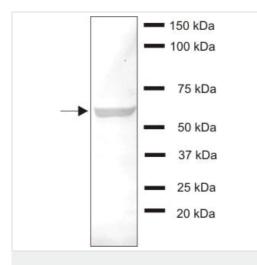
Secondary: Goat polyclonal to mouse IgG light chain specific (HRP) at 1/20,000 dilution.

Band: 70kDa, non specific bands - 50kDa: We are unsure as to the identity of this extra band; Hsp70

Immunohistochemistry analysis using ab47454 (1:100) staining paraffin-embedded mouse backskin. Stained for 1 hour at RT. Secondary Antibody: FITC Goat Anti-Mouse (green) at 1:50 for 1



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hsp70 antibody [N27F3-4] (ab47454)



Anti-Hsp70 antibody [N27F3-4] (ab47454) at 1/500 dilution + Apteronotus leptorhynchus brain tissue lysate at 50 μg

Secondary

hour at RT.

Alexa Fluor® 488-conjugated Goat anti-mouse IgG polyclonal at 1/1000 dilution

Performed under reducing conditions.

Predicted band size: 70 kDa Observed band size: 70 kDa

Exposure time: 5 minutes

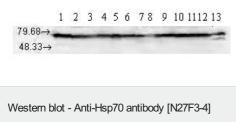
All lanes : Anti-Hsp70 antibody [N27F3-4] (ab47454) at 1 µg/ml

Lane 1 : Cell lysate prepared from human A431 cell line

- Lane 2 : Cell lysate prepared from human A549 cell line
- Lane 3 : Cell lysate prepared from human HCT116 cell line
- Lane 4 : Cell lysate prepared from human Hela cell line
- Lane 5 : Cell lysate prepared from human HEK293 cell line
- Lane 6 : Cell lysate prepared from human HepG2 cell line
- Lane 7 : Cell lysate prepared from human HL-60 cell line
- Lane 8 : Cell lysate prepared from human HUVEC cell line

Western blot - Anti-Hsp70 antibody [N27F3-4] (ab47454)

This image is courtesy of an anonymous Abreview

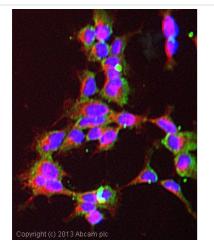


(ab47454)

Lane 9 : Cell lysate prepared from human Jurkat cell line Lane 10 : Cell lysate prepared from human MCF7 cell line Lane 11 : Cell lysate prepared from human PC3 cell line Lane 12 : Cell lysate prepared from human T98G cell line Lane 13 : Lysate prepared from rat brain tissue

Lysates/proteins at 10 µg per lane.

Predicted band size: 70 kDa



Copyright (e) 2013 Abcam plc Immunocytochemistry/ Immunofluorescence - Anti-Hsp70 antibody [N27F3-4] (ab47454)

ICC/IF image of ab47454 stained Hek293 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab47454, 5µg/ml) overnight at +4°C. The secondary antibody (green) was **ab96879**, DyLight® 488 goat anti-mouse IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

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