abcam

ab59457

Anti-Hsp60 antibody [LK-1] - Mitochondrial Marker

Lab Essentials

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	Preservative: 0.09% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine)
Form	Liquid
Clonality	Monoclonal
Immunogen	Recombinant Full Length Protein corresponding to Human HSPD1. Database link P10809
Clone number	LK-1
Purification technique	Affinity purification Protein G
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-
Notes	The antigen retrieval used on the human colon carcinoma IHC was 10% citric acid buffer, 121°C for 20 minutes.

Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Expected

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

IP

Tested

Species	Rat
Dilution info	5 µg/mL
Notes	-

Expected

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

Flow Cyt

Tested

Species	Human
Dilution info	1 µg for 10^6 Cells
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Expected

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

WB

Tested

Species	Rat
Dilution info	0.05 µg/mL
Notes	-

Species	Mouse
Dilution info	0.05 µg/mL
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

Expected

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

Target data

See full target information HSPD1

Function	Chaperonin implicated in mitochondrial protein import and macromolecular assembly. Together with Hsp10, facilitates the correct folding of imported proteins. May also prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions in the mitochondrial matrix (PubMed:11422376, PubMed:1346131). The functional units of these chaperonins consist of heptameric rings of the large subunit Hsp60, which function as a back-to-back double ring. In a cyclic reaction, Hsp60 ring complexes bind one unfolded substrate protein per ring, followed by the binding of ATP and association with 2 heptameric rings of the co-chaperonin Hsp10. This leads to sequestration of the substrate protein in the inner cavity of Hsp60 where, for a certain period of time, it can fold undisturbed by other cell components. Synchronous hydrolysis of ATP in all Hsp60 subunits results in the dissociation of the chaperonin rings and the release of ADP and the folded substrate protein (Probable).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Storage information	Stable for 12 months at -20°C

Supplementary info

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

Activity summary	Hsp60 also known as HSPD1 and heat shock protein 60 is a significant chaperonin with a molecular weight of approximately 60 kDa. It resides predominantly in the mitochondria and functions to assist in the proper folding of proteins preventing their aggregation. Hsp60 is expressed mainly in cells with high metabolic activity. Its presence as a mitochondrial marker highlights its essential role in maintaining cellular homeostasis and function.
Biological function summary	The protein ensures mitochondrial protein stability by facilitating the refolding of misfolded proteins and cooperating with other chaperonins like Hsp10. Hsp60 participates in forming a complex with these proteins to create a conducive environment for protein folding. It plays a part in regulating mitochondrial homeostasis impacting cell survival and apoptosis processes.
Pathways	Hsp60 links to the ATP synthesis and apoptosis pathways showcasing its importance as a mitochondrial marker. It interacts with proteins like caspase-3 to modulate cell death mechanisms highlighting its influence beyond simple protein folding. In the ATP synthesis pathway it contributes indirectly to energy production by maintaining mitochondrial function.

Associated diseases and disorders	Hsp60 shows a connection to neurodegenerative diseases and cancer. Altered Hsp60 levels correlate with increased apoptosis in neurodegenerative conditions like Alzheimer's disease. Additionally in cancer interactions with proteins such as AKT suggest its potential role in cell proliferation and survival. Understanding its role could aid in the development of therapeutic interventions targeting mitochondrial dysfunction.

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

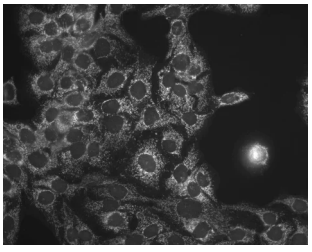
We do not recommend this combination. It is not covered by our product promise.

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.

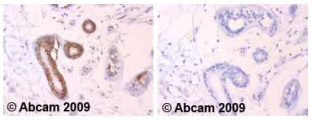
8 product images



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [LK-1] - Mitochondrial Marker (ab59457)

Hsp60 Immunocytochemistry/ Immunofluorescence staining of HaCaT cells using mouse Anti-Hsp60 antibody

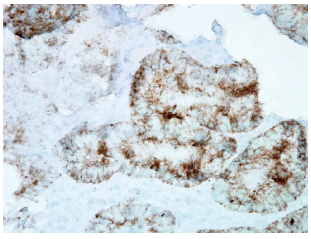
Immunofluorescent analysis of 100% methanol-fixed HaCaT cells labeling Hsp60 with ab59457 at 1/100 dilution. Secondary antibody: FITC Goat Anti-Mouse (green) at 1/50 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp60 antibody [LK-1] - Mitochondrial Marker (ab59457)

Hsp60 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-Hsp60 antibody

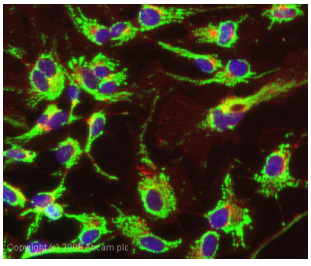
ab59457 staining human normal skin tissue. Staining is localised to mitochondria.
Left panel: with primary antibody at 2 ug/ml. Right panel: isotype control.
Sections were stained using an automated system DAKO Autostainer Plus , at room temperature. Sections were rehydrated and antigen retrieved with 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-Hsp60 antibody [LK-1] - Mitochondrial Marker (ab59457)

Hsp60 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-Hsp60 antibody

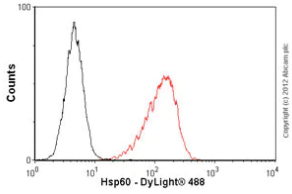
Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue labelling Hsp60 with ab59457 at 1/100,000 dilution for 1 hour at room temperature, followed by secondary antibody Goat Anti-Mouse (Biotin) at 1/2000 dilution for 1 hour at room temperature. Predominantly mitochondrial staining on Inflammatory cells in human colon carcinoma is observed. Counter stained with Hematoxylin.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [LK-1] - Mitochondrial Marker (ab59457)

Hsp60 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Hsp60 antibody

ICC/IF image of ab59457 stained HepG2 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 (ab59457, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 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.

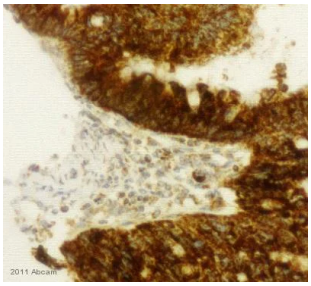


Flow Cytometry - Anti-Hsp60 antibody [LK-1] - Mitochondrial Marker (ab59457)

Hsp60 Flow Cytometry staining using mouse Anti-Hsp60 antibody

Overlay histogram showing HeLa cells stained with ab59457 (red line). The cells were fixed with 80% methanol (5 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 (ab59457, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

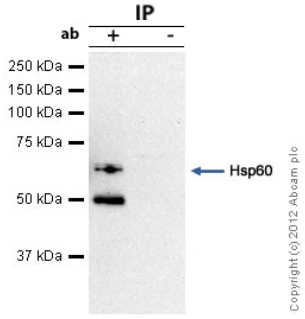


This is image is courtesy of a customer review submitted by Aamir Ahmed.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp60 antibody [LK-1] - Mitochondrial Marker (ab59457)

Hsp60 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-Hsp60 antibody

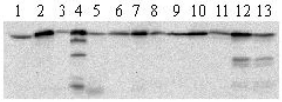
ab59457 staining Hsp60 in Human malignant colon tissue sections by Immunohistochemistry. Antigen retrieval was by heat mediation using retrieval buffer, pH6. Cells were fixed with paraformaldehyde and blocked with 3% H_2O_2 for 10 minutes at 22°C. Samples were incubated with primary antibody at 1/2000 dilution for 2 hours at 22°C. A HRP conjugated goat polyclonal was used as a secondary antibody.



Immunoprecipitation - Anti-Hsp60 antibody [LK-1] - Mitochondrial Marker (ab59457)

Hsp60 was immunoprecipitated using 0.5mg Rat Brain tissue lysate, 5µg of Mouse monoclonal to Hsp60 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 Brain 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 70ºC; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab59457.
Secondary: Goat polyclonal to mouse IgG light chain specific (HRP) at 1/20,000 dilution.
Band: 60kDa; Hsp60: non specific bands - 50kDa: We are unsure as to the identity of this extra band.
All lanes:
Immunoprecipitation - Anti-Hsp60 antibody [LK-1] - Mitochondrial Marker (ab59457)

Predicted band size: 61 kDa



Western blot - Anti-Hsp60 antibody [LK-1] - Mitochondrial Marker (ab59457)

Hsp60 Western blot staining using mouse Anti-Hsp60 antibody

All lanes:
Western blot - Anti-Hsp60 antibody [LK-1] - Mitochondrial Marker (ab59457) at 0.05 µg/mL

Lane 1:
Rat Brain tissue lysates at 10 µg

Lane 2:
Rat Heart tissue lysates at 10 µg

Lane 3:

Rat Kidney tissue lysates at 10 µg

Lane 4:
Rat Liver tissue lysates at 10 µg

Lane 5:
Rat Lung tissue lysates at 10 µg

Lane 6:
Rat Pancreas tissue lysates at 10 µg

Lane 7:
Rat skeletal muscle tissue lysate at 10 µg

Lane 8:
Rat Spleen tissue lysate at 10 µg

Lane 9:
Rat Testes tissue lysate at 10 µg

Lane 10:
Rat Thymus tisuue lysate at 10 µg

Lane 11:
Cell lysates prepared from rat heart H9C2 cells at 10 µg

Lane 12:
Cell lysates prepared from mouse NIH3T3 cells at 10 µg

Lane 13:
Cell lysates prepared from mouse Pam212 cells at 10 µg

Secondary

All lanes:
HRP-conjugated goat polyclonal to mouse IgG1 at 1/10 dilution

Predicted band size: 61 kDa

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