abcam

ab5478

Anti-Hsp60 antibody [4B9/89] - Mitochondrial Marker

Lab Essentials

Key facts

Isotype	IgG2a
Host species	Mouse
Storage buffer	Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Full Length Protein corresponding to Human HSPD1. The exact immunogen used to generate this antibody is proprietary information. Database link P10809
Clone number	4B9/89
Purification technique	Affinity purification Protein A
Epitope	Epitope mapping studies using human Hsp 60 deletion mutants suggest that this antibody binds either between amino acids 335-366 or 484-547.
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	1/200
Notes	-

Expected

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

IP

Tested

Species	Human
Dilution info	0.5-2 µg/mg of lysate
Notes	-

Expected

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

Flow Cyt

Tested

Species	Human
Dilution info	1-20 µg/mL
Notes	-

Expected

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

WB

Tested

Species	Human
Dilution info	1/100 - 1/1000
Notes	Detects a band of approximately 60 kDa representing Hsp 60 from human blood samples.

Expected

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

ICC/IF

Tested

Species	Mouse
Dilution info	1/50 - 1/60
Notes	-

Species	Human
Dilution info	1/50 - 1/60
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 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	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

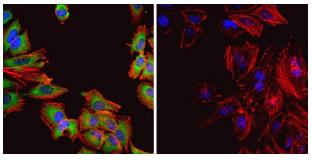
We do not recommend this combination. It is not covered by our product promise.

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.

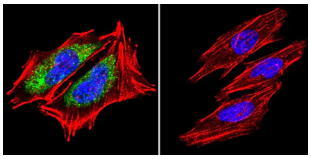
12 product images



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [4B9/89] - Mitochondrial Marker (ab5478)

Hsp60 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Hsp60 antibody

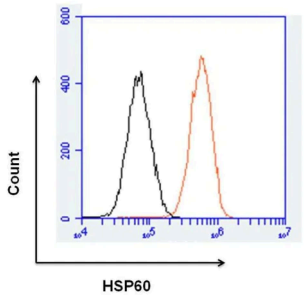
Immunocytochemistry/Immunofluorescence analysis of Hsp60 (green) in HeLa cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with (left panel) or without (right panel) ab5478 at a dilution of 1:50 for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488 goat-anti-mouse IgG secondary antibody at a dilution of 1:400 for 30 minutes at room temperature. F-Actin (red) was stained with Dylight 554 phalloidin, and nuclei (blue) were stained with Hoechst 33342 dye. Images were taken at 20X magnification.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [4B9/89] - Mitochondrial Marker (ab5478)

Hsp60 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Hsp60 antibody

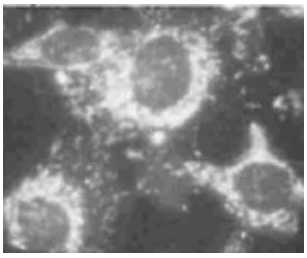
Immunocytochemistry/Immunofluorescence analysis of Hsp60 in A2058 Cells. Hsp60 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with ab5478 at a dilution of 1:200 over night at 4 °C, washed with PBS and incubated with a DyLight-488 conjugated goat anti-mouse IgG secondary antibody. Images were taken at 60X magnification.



Flow Cytometry - Anti-Hsp60 antibody [4B9/89] - Mitochondrial Marker (ab5478)

Hsp60 Flow Cytometry staining using mouse Anti-Hsp60 antibody

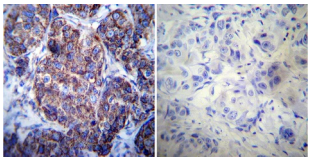
Flow cytometry analysis of HSP60 was done on HeLa cells. Cells were fixed, permeabilized and stained with a HSP60 mouse monoclonal antibody (orange histogram) or a mouse IgG2a isotype control (black histogram) at a dilution of 10 µg/mL. After incubation for 1 hour on ice, the cells were labeled with a Goat anti-Mouse IgG Secondary Antibody, DyLight 650 conjugate at 1/50 dilution for 1 hour on ice. A representative 10,000 cells were acquired and analyzed for each sample.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [4B9/89] - Mitochondrial Marker (ab5478)

Hsp60 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Hsp60 antibody

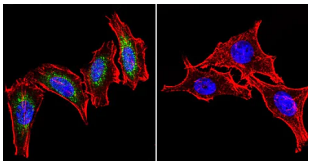
Immunolocalization of Hsp 60 in human endothelial cells using ab5478.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp60 antibody [4B9/89] - Mitochondrial Marker (ab5478)

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

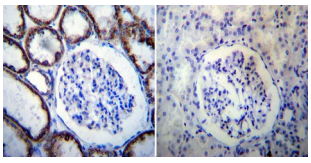
Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized human Breast carcinoma tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:50 with a mouse monoclonal antibody recognizing Heat Shock Protein 60 (ab5478) 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-Hsp60 antibody [4B9/89] - Mitochondrial Marker (ab5478)

Hsp60 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Hsp60 antibody

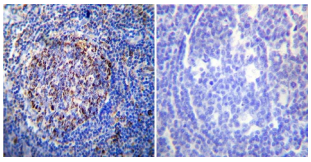
Immunocytochemistry/Immunofluorescence analysis of Hsp60 in Hela Cells. Hsp60 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were incubated without (control) or with ab5478 at a dilution of 1:100 over night at 4°C, washed with PBS and incubated with a DyLight-488 conjugated goat anti-mouse secondary antibody. Images were taken at 60X magnification.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp60 antibody [4B9/89] - Mitochondrial Marker (ab5478)

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

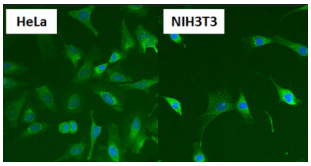
Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized human Kidney tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:100 with a mouse monoclonal antibody recognizing Heat Shock Protein 60 (ab5478) 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-Hsp60 antibody [4B9/89] - Mitochondrial Marker (ab5478)

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

Immunohistochemistry was performed on both normal and cancer 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 1:100 with a mouse monoclonal antibody recognizing Heat Shock Protein 60 (ab5478) 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-Hsp60 antibody [4B9/89] - Mitochondrial Marker (ab5478)

Hsp60 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Hsp60 antibody

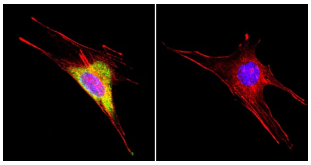
Immunocytochemistry/Immunofluorescence analysis of Hsp60 (green) in HeLa and NIH3T3 cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with ab5478 at a dilution of 1:50 for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488 goat-anti-mouse IgG 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-Hsp60 antibody [4B9/89] - Mitochondrial Marker (ab5478)

Immunoprecipitation of Hsp60 was performed on HeLa cells. Antigen:antibody complexes were formed by incubating 500µg whole cell lysate with 2µg of HSP60 monoclonal antibody (ab5478) overnight on a rocking platform at 4°C. The immune complexes were captured on 50µl Protein Agarose washed extensively and eluted with Buffer. Samples were then resolved on a 4-20% Tris-HCl polyacrylamide gel then transferred to a PVDF membrane and blocked with 5% BSA/TBST for at least 1 hour. The membrane was probed with a HSP60 monoclonal antibody (ab5478) at a dilution of 1:1000 overnight rotating at 4°C, washed in TBSTand probed with Detection Reagent (HRP) at a dilution of 1:1000 for at least one hour. Chemiluminescent detection was performed.
All lanes:
Immunoprecipitation - Anti-Hsp60 antibody [4B9/89] - Mitochondrial Marker (ab5478)

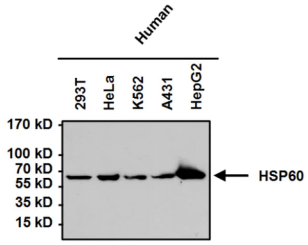
Predicted band size: 61 kDa



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [4B9/89] - Mitochondrial Marker (ab5478)

Hsp60 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Hsp60 antibody

Immunocytochemistry/Immunofluorescence analysis of Hsp60 in ATDC5 Cells. Hsp60 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were incubated without (control) or with ab5478 at a dilution of 1:100 over night at 4°C, washed with PBS and incubated with a DyLight-488 conjugated goat anti-mouse IgG secondary antibody. Images were taken at 60X magnification.



Western blot - Anti-Hsp60 antibody [4B9/89] - Mitochondrial Marker (ab5478)

Hsp60 Western blot staining using mouse Anti-Hsp60 antibody

All lanes:
Western blot - Anti-Hsp60 antibody [4B9/89] - Mitochondrial Marker (ab5478) at 1/1000 dilution

Lane 1:
293T cell lysate at 50 µg

Lane 2:
HeLa cell lysate at 50 µg

Lane 3:
K562 cell lysate at 50 µg

Lane 4:

A431 cell lysate at 50 µg

Lane 5:
HepG2 cell lysate at 50 µg

Secondary

All lanes:
HRP-conjugated goat anti-mouse IgG at 1/20000 dilution

Predicted band size: 61 kDa

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