abcam

Product datasheet

Anti-Hsp60 antibody [2E1/53] ab5479

2 References 13 Images

Overview

Product name	Anti-Hsp60 antibody [2E1/53]
Description	Mouse monoclonal [2E1/53] to Hsp60
Host species	Mouse
Specificity	ab5479 detects Hsp 60 from Human cells, tissues and recombinant protein preparations. This antibody displays no other protein or species cross-reactivity.
Tested applications	**Suitable for:** IHC-P, ICC/IF, IP, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human, African green monkey
Immunogen	Full length protein corresponding to Human Hsp60. Human placental Hsp60.
Epitope	Epitope mapping studies using human Hsp 60 deletion mutants suggest that this antibody binds between amino acids 211-288.
Positive control	WB: HeLa ,A431, HEK-293, HepG2, COS-7, NIH/3T3, THP1, K562, A549, HEK-293T, U-2 OS, NRK whole cell lysate. IHC-P: Human kidney and breast carcinoma tissue. ICC: U-251, A431, HeLa, NIH-3T3 cells. IP: HeLa whole cell lysates.
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

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 Constituents: 0.1% BSA, 99% PBS
Purity	Purified IgM
Clonality	Monoclonal

Clone number	2E1/53
Isotype	IgM

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab5479 in the following tested applications.

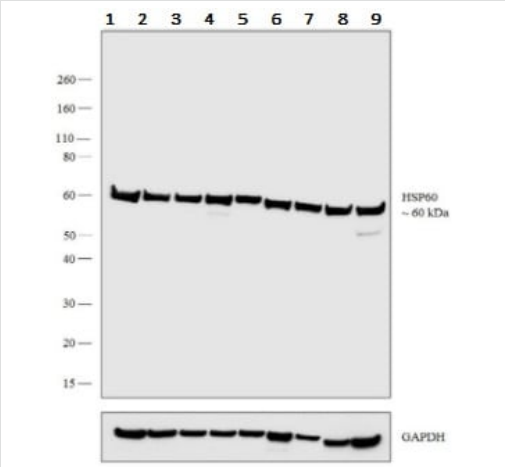
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		Use a concentration of 1 - 10 µg/ml.
ICC/IF		1/20 - 1/200.
IP		Use a concentration of 2 µg/ml.
WB		1/2000. Detects a band of approximately 58 kDa.

Target

Function	Implicated in mitochondrial protein import and macromolecular assembly. May facilitate 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.
Involvement in disease	Defects in HSPD1 are a cause of spastic paraplegia autosomal dominant type 13 (SPG13) [MIM:605280]. Spastic paraplegia is a degenerative spinal cord disorder characterized by a slow, gradual, progressive weakness and spasticity of the lower limbs. Defects in HSPD1 are the cause of leukodystrophy hypomyelinating type 4 (HLD4) [MIM:612233]; also called mitochondrial HSP60 chaperonopathy or MitCHAP-60 disease. HLD4 is a severe autosomal recessive hypomyelinating leukodystrophy. Clinically characterized by infantile-onset rotary nystagmus, progressive spastic paraplegia, neurologic regression, motor impairment, profound mental retardation. Death usually occurrs within the first two decades of life.
Sequence similarities	Belongs to the chaperonin (HSP60) family.
Cellular localization	Mitochondrion matrix.

Images



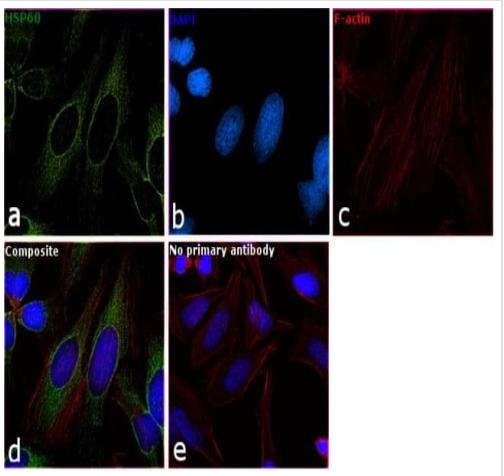
Western blot - Anti-Hsp60 antibody [2E1/53] (ab5479)

All lanes : Anti-Hsp60 antibody [2E1/53] (ab5479) at 1/2000 dilution

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : A431 (human epidermoid carcinoma cell line) whole cell lysate
Lane 3 : HEK-293 (h
uman epithelial cell line from embryonic kidney) whole cell lysate
Lane 4 : HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate
Lane 5 : COS-7 (african green monkey kidney fibroblast-like cell line) whole cell lysate
Lane 6 : NIH/3T3 (mouse embryonic fibroblast cell line) whole cell lysate
Lane 7 : THP1 (human monocytic leukemia cell line) whole cell lysate
Lane 8 : K562 (human chronic myelogenous leukemia lymphoblast cell line) whole cell lysate
Lane 9 : A549 (human lung carcinoma cell line) whole cell lysate

Lysates/proteins at 30 µg per lane.

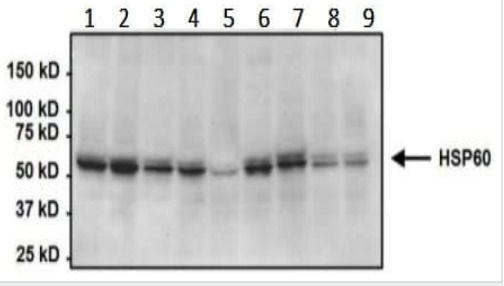
Secondary
All lanes : Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate at 1/4000 dilution



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [2E1/53] (ab5479)

Immunofluorescence analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling HSP60 at 1/200 dilution in 0.1% BSA, incubated at 4°C overnight, followed by Goat anti-Mouse IgG (H+L)/IgM (L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate at 1/2000 dilution for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI. F-actin (Panel c: red) was stained with Rhodamine Phalloidin at 1/300 dilution. Panel d represents the merged image showing mitochondrial localization. Panel e represents control cells with no primary antibody to assess background.

The cells (70% confluent log phase) were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 1% BSA for 1 hour at room temperature. The images were captured at 60X magnification.



Western blot - Anti-Hsp60 antibody [2E1/53] (ab5479)

All lanes : Anti-Hsp60 antibody [2E1/53] (ab5479) at 1 µg/ml (overnight at 4°C)

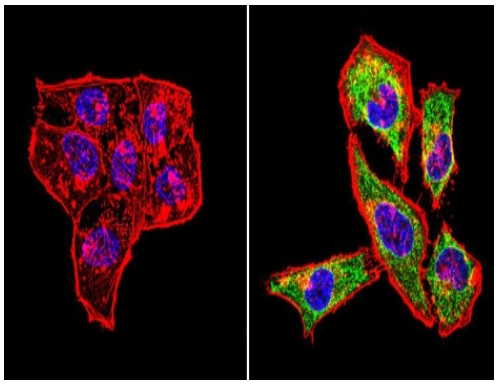
Lane 1 : HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate with 5% BSA/TBST for at least 1 hour
Lane 2 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with 5% BSA/TBST for at least 1 hour
Lane 3 : K562 (human chronic myelogenous leukemia lymphoblast cell line) whole cell lysate with 5% BSA/TBST for at least 1 hour
Lane 4 : A431 (human epidermoid carcinoma cell line) whole cell lysate with 5% BSA/TBST for at least 1 hour
Lane 5 : HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate with 5% BSA/TBST for at least 1 hour
Lane 6 : U-2 OS (human bone osteosarcoma epithelial cell line) whole cell lysate with 5% BSA/TBST for at least 1 hour
Lane 7 : COS-7 (african green monkey kidney fibroblast-like cell line) whole cell lysate with 5% BSA/TBST for at least 1 hour

Lane 8 : NIH/3T3 (mouse embryonic fibroblast cell line) whole cell lysate with 5% BSA/TBST for at least 1 hour
Lane 9 : NRK (rat kidney cell line) whole cell lysate with 5% BSA/TBST for at least 1 hour

Lysates/proteins at 50 µg per lane.

Secondary
All lanes : Goat anti-mouse IgM secondary antibody at 1/20000 dilution

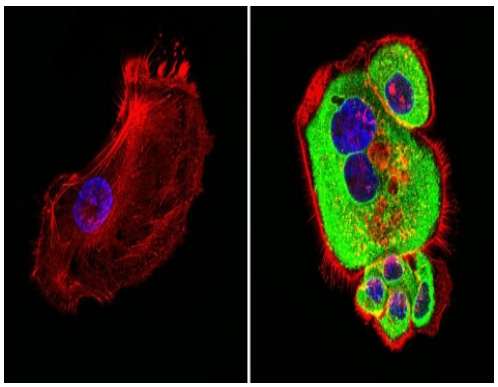
Western blot analysis of various cell lines (50µg/lane) labeling Hsp60 with ab5479 at 1µg/ml.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [2E1/53] (ab5479)

Immunofluorescence analysis of Hsp60 (green) in HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling Hsp60 with ab5479 at 1/200 dilution and incubated overnight in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody for 45 minutes at room temperature in the dark. F-actin (red) was stained with a fluorescent phalloidin and nuclei (blue) were stained with DAPI.

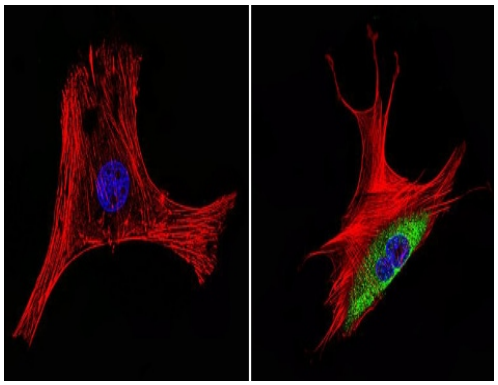
Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes at room temperature and blocked with 3% BSA-PBS for 30 minutes at room temperature. Images were taken at a 60X magnification.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [2E1/53] (ab5479)

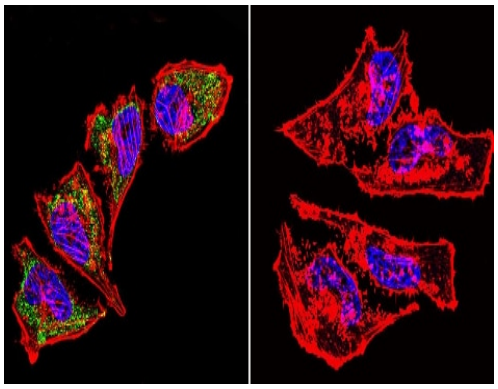
Immunofluorescence analysis of A431 (human epidermoid carcinoma cell line) cells labeling Hsp60 (green) with ab5479 at 1/100 dilution and incubated overnight in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody for 45 minutes at room temperature in the dark. F-actin (red) was stained with a fluorescent phalloidin and nuclei (blue) were stained with DAPI.

Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes at room temperature and blocked with 3% BSA-PBS for 30 minutes at room temperature. Images were taken at a 60X magnification.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [2E1/53] (ab5479)

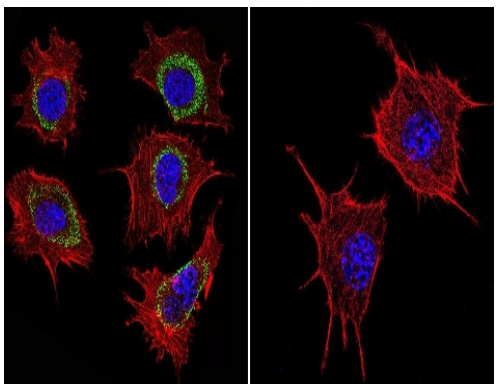
Immunofluorescence analysis of NIH/3T3 (mouse embryonic fibroblast cell line) cells labeling Hsp60 (green) with ab5479 at 1/20 dilution and incubated overnight in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody for 45 minutes at room temperature in the dark. F-actin (red) was stained with a fluorescent phalloidin and nuclei (blue) were stained with DAPI. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes at room temperature and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with Images were taken at a 60X magnification.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [2E1/53] (ab5479)

Immunofluorescence analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling Hsp60 (green) with ab5479 at 1/100 dilution or without overnight at 4 C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown.

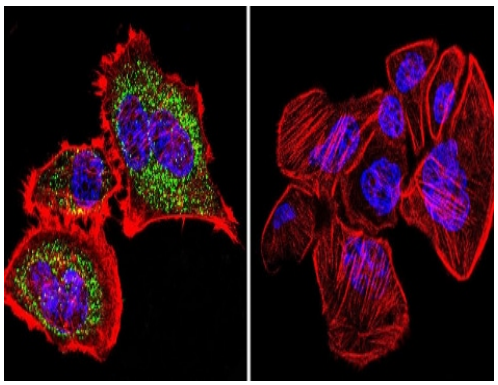
Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Images were taken at 60X magnification.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [2E1/53] (ab5479)

Immunofluorescence analysis of NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling Hsp60 (green) with ab5479 at 1/100 dilution or without (negative control) overnight at 4°C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown.

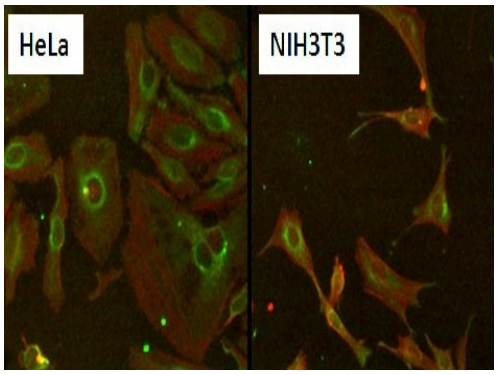
Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Images were taken at 60X magnification.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [2E1/53] (ab5479)

Immunofluorescence analysis of U-251 MG (formally U-373 MG) (human brain glioma cell line) cells labeling Hsp60 with ab5479 at 1/200 dilution overnight at 4°C, washed with PBS, followed by DyLight-488 conjugated secondary antibody.

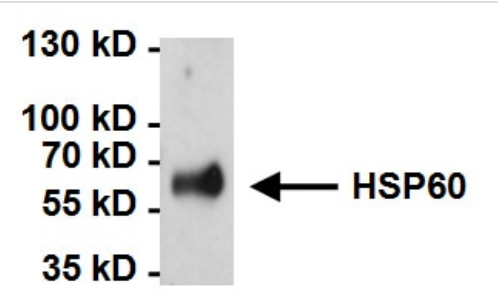
Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with ab5479. Heat Shock Protein 60 (Hsp60) staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [2E1/53] (ab5479)

Immunofluorescence analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) and NIH/3T3 (mouse embryonic fibroblast cell line) cells labeling HSP60 (green) with ab5479 at 10ug/ml for at least 1 hour at room temperature. Cells were washed with PBS and incubated with a fluorescently labeled goat anti-mouse IgM secondary antibody at 1/400 dilution for 30 minutes at room temperature. F-Actin (red) was stained with DyLight 554 Phalloidin and nuclei (blue) were stained with Hoechst 33342 dye.

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. Images were taken at 20X magnification.



Immunoprecipitation - Anti-Hsp60 antibody [2E1/53] (ab5479)

Immunoprecipitation of Hsp60 was performed on HeLa (human epithelial cell line from cervix adenocarcinoma) cells. Antigen-antibody complexes were formed by incubating 500ug of whole cell lysate with 2ug of ab5479 overnight on a rocking platform at 4°C. The immune complexes were captured on 50ul Protein A/G Plus Agarose, washed extensively, and eluted with 5X Lane Marker Reducing Sample Buffer. Samples were resolved on a 4-20% Tris-HCl polyacrylamide gel, transferred to a PVDF membrane, and blocked with 5% BSA/TBST for at least 1 hour. The membrane was probed with ab5479 at a concentration of 1ug/ml overnight rotating at 4°C, washed in TBST, and probed with a goat anti-mouse IgM secondary antibody at a dilution of 1:20,000 for at least 1 hour. Chemiluminescent detection performed using SuperSignal West Dura.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp60 antibody [2E1/53] (ab5479)

Immunohistochemiscal analysis of both normal and cancer biopsies of deparaffinized human breast carcinoma tissues labeling Hsp60 with ab5479 at 1/20 dilution 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.

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.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp60 antibody [2E1/53] (ab5479)

Immunohistochemiscal analysis of both normal and cancer biopsies of deparaffinized human kidney tissue labeling Hsp60 with 1/100 dilution 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.

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.

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