

Anti-Hsp60 antibody [2E1/53] ab5479

2 References **13 图像**

概述

产品名称	Anti-Hsp60抗体[2E1/53]
描述	小鼠单克隆抗体[2E1/53] to Hsp60
宿主	Mouse
特异性	ab5479 detects Hsp 60 from Human cells, tissues and recombinant protein preparations. This antibody displays no other protein or species cross-reactivity.
经测试应用	适用于: IHC-P, ICC/IF, IP, WB
种属反应性	与反应: Mouse, Rat, Human, African green monkey
免疫原	Full length protein corresponding to Human Hsp60. Human placental Hsp60.
表位	Epitope mapping studies using human Hsp 60 deletion mutants suggest that this antibody binds between amino acids 211-288.
阳性对照	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.
常规说明	<p>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.</p> <p>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</p>

性能

形式	Liquid
存放说明	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.
存储溶液	Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 99% PBS
纯度	Purified IgM
克隆	单克隆

克隆编号2E1/53
同种型IgM

应用

The Abpromise guarantee **Abpromise™**承诺保证使用ab5479于以下的经测试应用

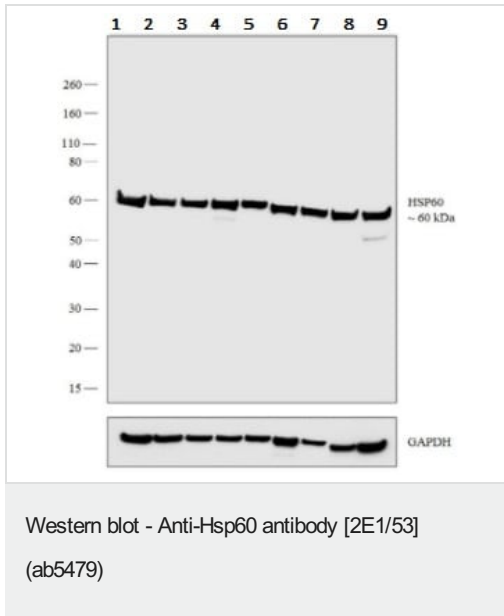
“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
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.

靶标

功能	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.
疾病相关	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 occurs within the first two decades of life.
序列相似性	Belongs to the chaperonin (HSP60) family.
细胞定位	Mitochondrion matrix.

图片



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 (human 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

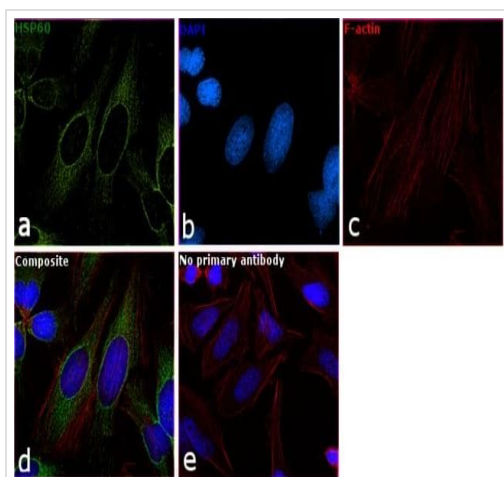
Lane 8 : K562 (human chronic myelogenous leukemia lymphoblast cell line) whole cell lysate

Lane 9 : A549 (human lung carcinoma cell line) whole cell lysate

Lysates/proteins at 30 µg per lane.

Secondary

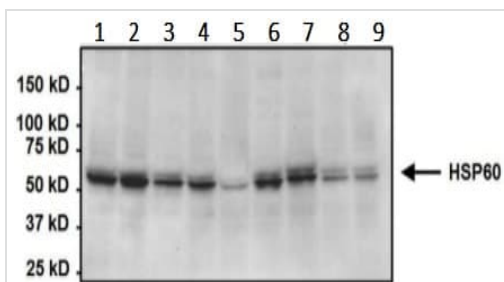
All lanes : Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate at 1/4000 dilution



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

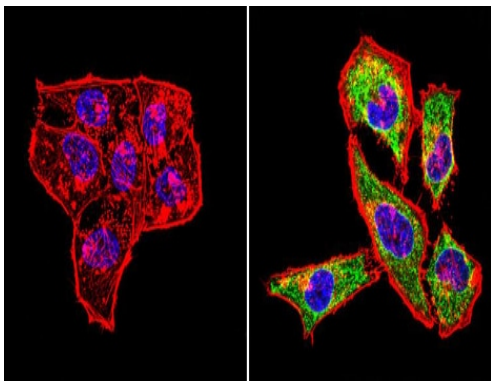
Lane 9 : NRK (rat kidney cell line) whole cell lysate with 5% BSA/TBST for at least 1 hour

Lysates/proteins at 50 µg per lane.

Secondary

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

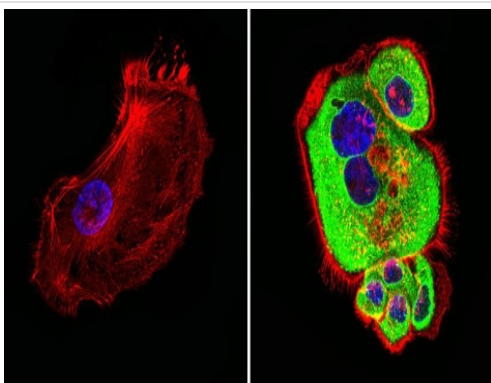
Western blot analysis of various cell lines (50µg/lane) labeling Hsp60 with ab5479 at 1µg/ml.



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.

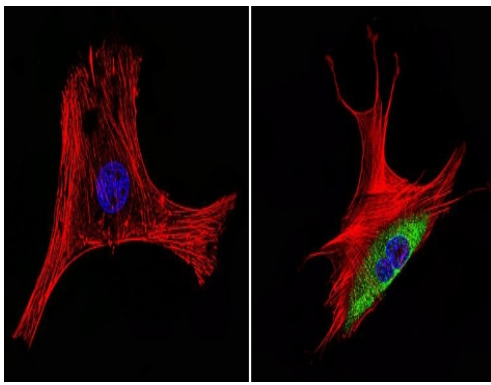
Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes at room temperature and blocked with 3% BSA-PBS for 30 minutes at room temperature. Images were taken at a 60X magnification.



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

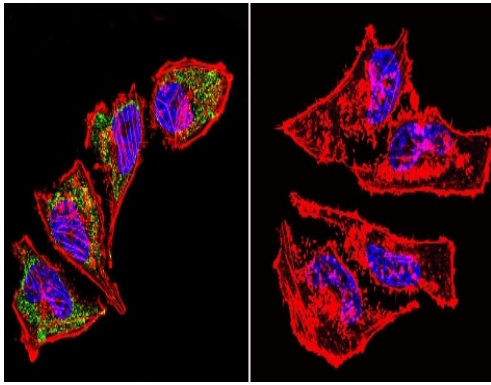
Immunofluorescence analysis of A431 (human epidermoid carcinoma cell line) cells labeling Hsp60 (green) with ab5479 at 1/100 dilution and incubated overnight in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody for 45 minutes at room temperature 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)

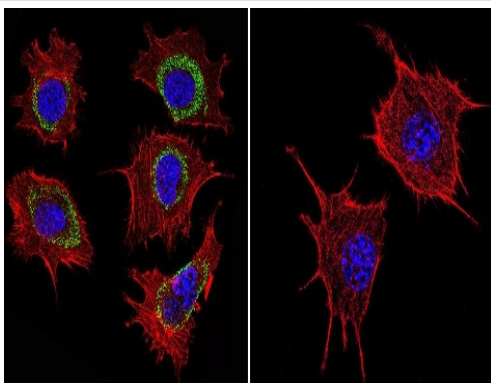
Immunofluorescence analysis of NIH/3T3 (mouse embryonic fibroblast cell line) cells labeling Hsp60 (green) with ab5479 at 1/20 dilution and incubated overnight in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody for 45 minutes at room temperature 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.

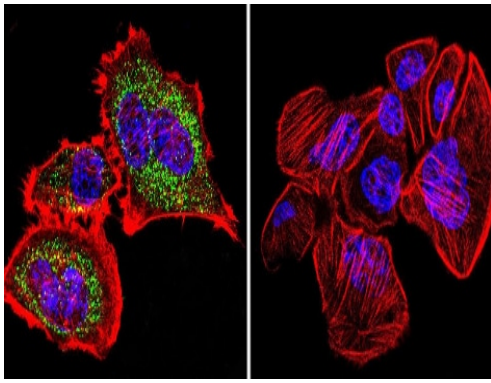
Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Images were taken at 60X magnification.



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.

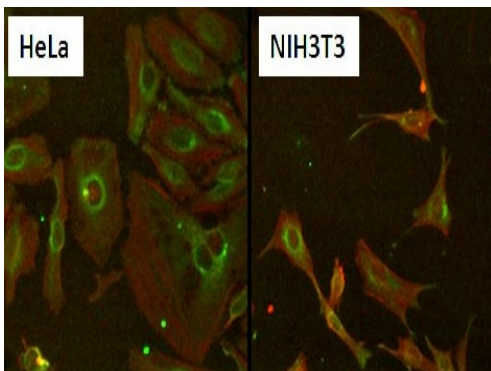
Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Images were taken at 60X magnification.



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.

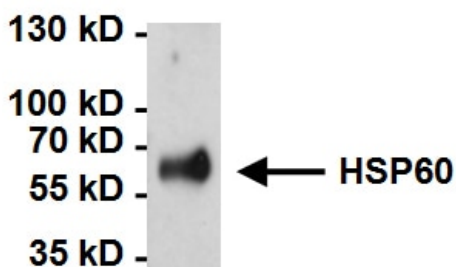
Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with ab5479. Heat Shock Protein 60 (Hsp60) staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.



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)

Immunohistochemical 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)

Immunohistochemical 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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