abcam

Product datasheet

Anti-Hsp60 antibody [4B9/89] ab5478

3 References 12 图像

概述

产品名称	Anti-Hsp60抗体[4B9/89]
描述	小鼠单克隆抗体[4B9/89] to Hsp60
宿主	Mouse
经测试应用	**适用于:** Flow Cyt, IHC-P, ICC/IF, IP, WB
种属反应性	**与反应:** Mouse, Human
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 either between amino acids 335-366 or 484-547.
常规说明	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

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	Constituent: 100% PBS
纯度	Protein A purified
克隆	单克隆
克隆编号	4B9/89
同种型	IgG2a

应用

The Abpromise guarantee	**Abpromise™承诺保证使用ab5478于以下的经测试应用**

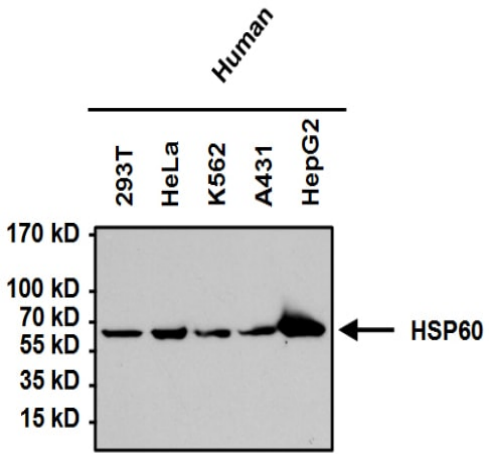
“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt		Use a concentration of 1 - 20 µg/ml.
IHC-P		1/200. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/50 - 1/60.
IP		Use at 2-0.5 µg/mg of lysate.
WB		1/100 - 1/1000. Detects a band of approximately 60 kDa. Detects a band of approximately 60 kDa representing Hsp 60 from human blood samples.

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 occurrs 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



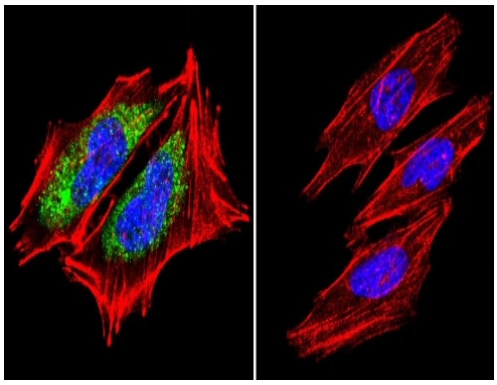
Western blot - Anti-Hsp60 antibody [4B9/89] (ab5478)

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

Lane 1 : 293T cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : K562 cell lysate
Lane 4 : A431 cell lysate
Lane 5 : HepG2 cell lysate

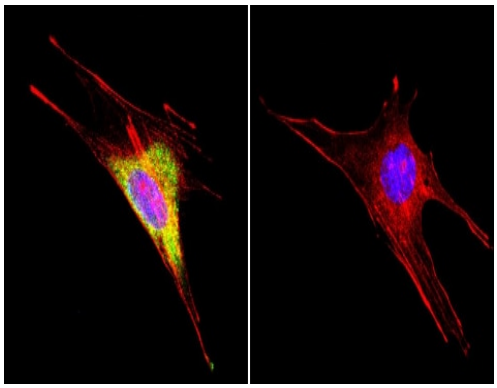
Lysates/proteins at 50 µg per lane.

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



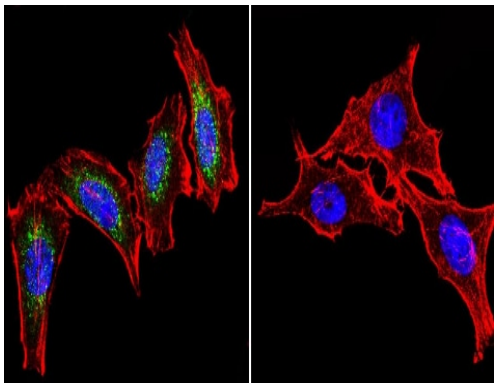
Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [4B9/89] (ab5478)

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.



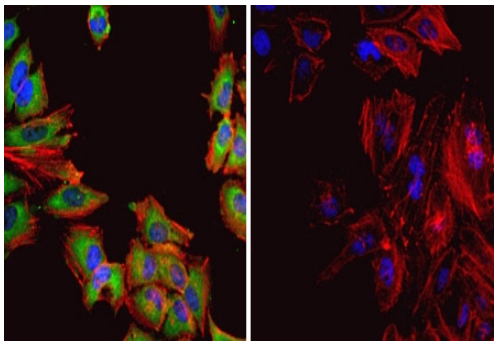
Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [4B9/89] (ab5478)

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.



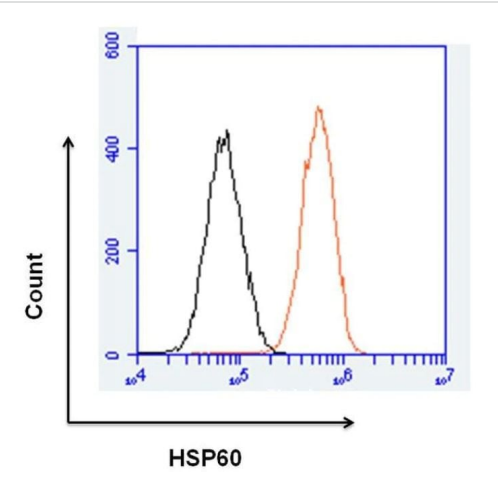
Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [4B9/89] (ab5478)

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.



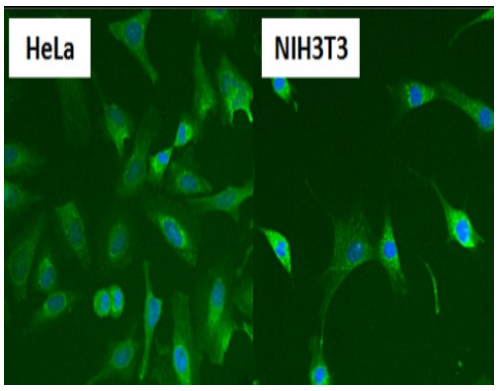
Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [4B9/89] (ab5478)

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.



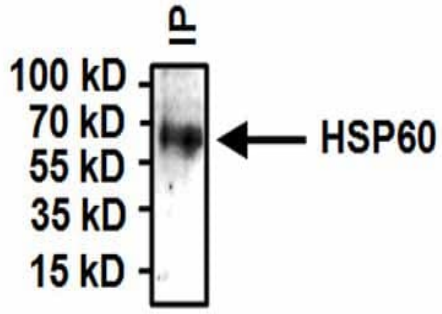
Flow Cytometry - Anti-Hsp60 antibody [4B9/89] (ab5478)

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.



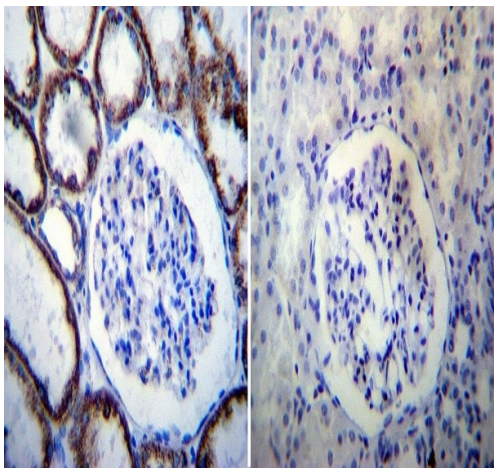
Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [4B9/89] (ab5478)

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.



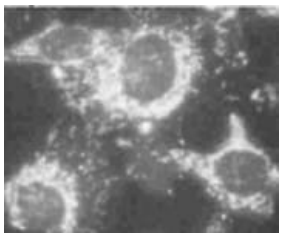
Immunoprecipitation - Anti-Hsp60 antibody [4B9/89] (ab5478)

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.



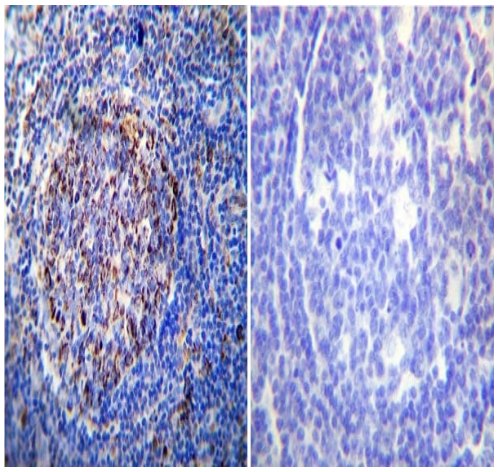
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp60 antibody [4B9/89] (ab5478)

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.



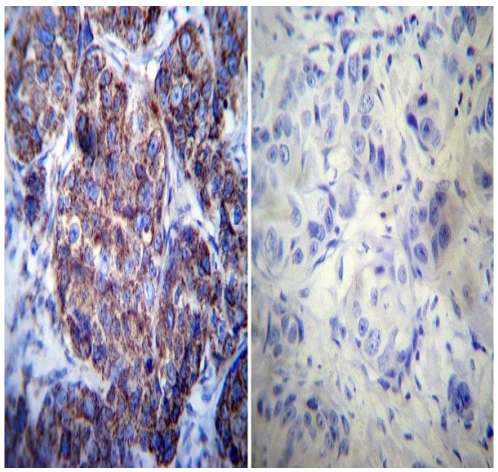
Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [4B9/89] (ab5478)

Immunolocalization of Hsp 60 in human endothelial cells using ab5478.



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

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.



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

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.

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