abcam

Product datasheet

Anti-Hsp60 antibody ab46798

★★★★★ 13 Abreviews 114 References 16 Images

Overview

Product name Anti-Hsp60 antibody

Description Rabbit polyclonal to Hsp60

Host species Rabbit

Specificity Replenishment batches of our polyclonal antibody, ab46798 are tested in WB. Previous batches

were additionally validated in Flow Cyt, ICC/IF, IHC-P and IP. These applications are still expected to work and are covered by our Abpromise guarantee. You may also be interested in

our alternative recombinant antibody, ab190828.

Tested applications Suitable for: WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra)

Species reactivity Reacts with: Mouse, Rat, Human, Pig

Predicted to work with: Chicken, Hamster, Cow, Arabidopsis thaliana, Chlamydomonas reinhardtii, Orangutan, Secale cereale, Brassica napus, Heliothis virescens, Syntrophobacter fumaroxidans, Methylacidiphilum infernorum, Thermodesulfovibrio yellowstonii, Pisum sativum

A

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control IHC: Human liver.

General notesThe 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.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

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Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee

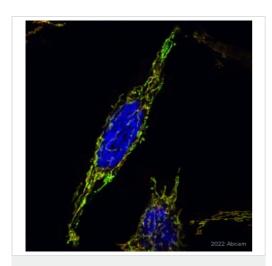
Our <u>Abpromise guarantee</u> covers the use of ab46798 in the following tested applications.

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

Application	Abreviews	Notes
WB	****(8)	1/20000. Detects a band of approximately 60 kDa (predicted molecular weight: 60 kDa).
IHC-P	**** <u>(2)</u>	1/100.
ICC/IF	**** <u>(1)</u>	Use a concentration of 5 - 10 µg/ml.
IP	★★★★☆ (1)	1/40.
Flow Cyt (Intra)		1/30. ab171870 - Rabbit polyclonal lgG, is suitable for use as an isotype control with this antibody.

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.	

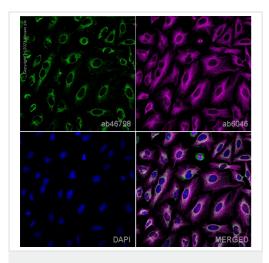
Images



Immunocytochemistry - Anti-Hsp60 antibody (ab46798)

This image is courtesy of an Abreview submitted by Suleyman Bozkurt

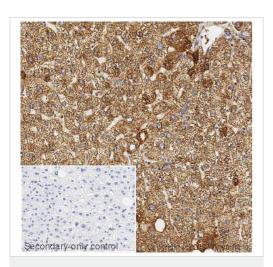
Immunocytochemistry analysis of paraformaldehyde-fixed 0.3% Triton X permeabilized HeLa FlipIn Cell line staining with ab46798 at 1/200 dilution. Secondary antibody was Alexa fluor™ 488 goat anti-rabbit lgG H+L at 1/700 dilution. Samples were incubated with the primary antibody with blocking buffer (5% FBS in PBS) for 2 hours at 24°C. Blocking was done using 5% serum for 1 hour at 24°C.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody (ab46798)

ab46798 staining Hsp60 in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab46798 at 1µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse lgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour magenta). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min). Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

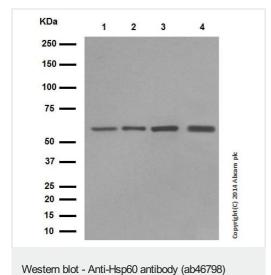


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hsp60 antibody (ab46798)

IHC image of Hsp60 staining in a section of formalin-fixed paraffinembedded normal human liver* performed on a Leica Biosystems BOND® RX instrument. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab46798, 1ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



All lanes : Anti-Hsp60 antibody (ab46798) at 1/20000 dilution (purified)

Lane 1 : Mouse brain tissue lysate at 20 μg
Lane 2 : Mouse heart tissue lysate at 20 μg
Lane 3 : Mouse spleen tissue lysate at 20 μg
Lane 4 : Rat heart tissue lysate at 10 μg

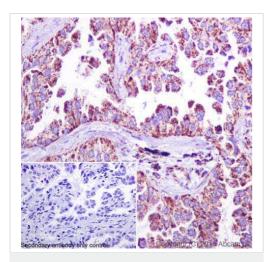
Secondary

All lanes : HRP conjugated goat anti-rabbit lgG (H+L) at 1/1000 dilution

Predicted band size: 60 kDa **Observed band size:** 60 kDa

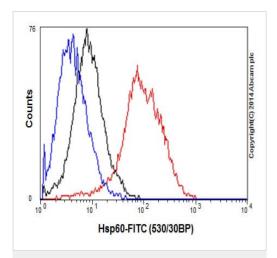
Blocking Buffer: 5% NFDM/TBST

Dilution Buffer: 5% NFDM/TBST



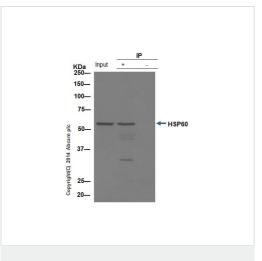
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hsp60 antibody (ab46798)

Immunohistochemical staining of paraffin embedded human lung adenocarcinoma with purified ab46798 at a working dilution of 1 in 100. The secondary antibody used is ab97051 Goat Anti-Rabbit IgG H&L (HRP) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Flow Cytometry (Intracellular) - Anti-Hsp60 antibody (ab46798)

Overlay histogram showing HeLa cells fixed in 2% PFA and stained with purified ab46798 at a dilution of 1 in 150 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 150. Rabbit monoclonal IgG was used as an isotype control (black) and cells without incubation with the antibody were used as a negative control (blue line).

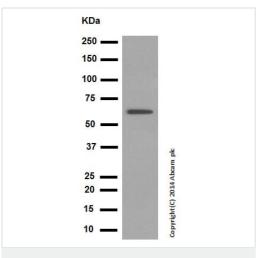


the secondary antibody (1/1500). Blocking buffer and concentration: 5% NFDM/TBST.

ab46798 (purified) at 1/40 immunoprecipitating Hsp60 in MCF7 (Lane 1). Lane 2 - PBS. For western blotting, a HRP-conjugated anti-rabbit lgG, specific to the non-reduced form of lgG was used as

Diluting buffer and concentration: 5% NFDM /TBST.

Immunoprecipitation - Anti-Hsp60 antibody (ab46798)



Anti-Hsp60 antibody (ab46798) at 1/20000 dilution (purified) + T47-D cell lysate at 10 µg

Secondary

HRP conjugated goat anti-rabbit lgG (H+L) at 1/1000 dilution

Predicted band size: 60 kDa Observed band size: 60 kDa

Western blot - Anti-Hsp60 antibody (ab46798)

Blocking Buffer: 5% NFDM/TBST Dilution Buffer: 5% NFDM/TBST

All lanes: Anti-Hsp60 antibody (ab46798) at 1/50000 dilution (purified)

Lane 1: MCF7 cell lysate Lane 2: SW480 cell lysate

Lysates/proteins at 20 µg per lane.

KDa 2 250 -100 -75 ---50 -37 -Copyright (C) 2014 Abcam plc 25 -20 -10 -

Western blot - Anti-Hsp60 antibody (ab46798)

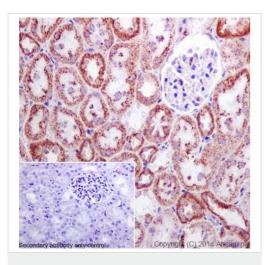
Secondary

All lanes: HRP conjugated goat anti-rabbit IgG (H+L) at 1/1000

dilution

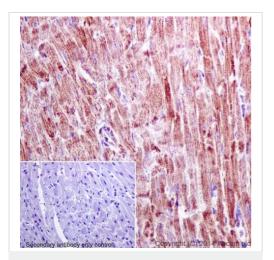
Predicted band size: 60 kDa Observed band size: 60 kDa Blocking Buffer: 5% NFDM/TBST

Dilution Buffer: 5% NFDM/TBST



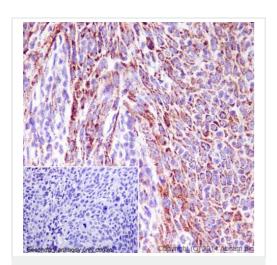
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hsp60 antibody (ab46798)

Immunohistochemical staining of paraffin embedded rat kidney with purified ab46798 at a working dilution of 1 in 100. The secondary antibody used is ab97051 Goat Anti-Rabbit IgG H&L (HRP) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



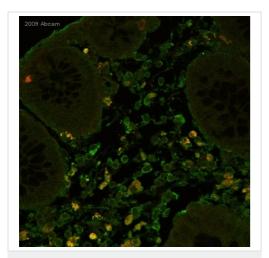
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hsp60 antibody (ab46798)

Immunohistochemical staining of paraffin embedded mouse cardiac muscle with purified ab46798 at a working dilution of 1 in 100. The secondary antibody used is ab97051 Goat Anti-Rabbit IgG H&L (HRP) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



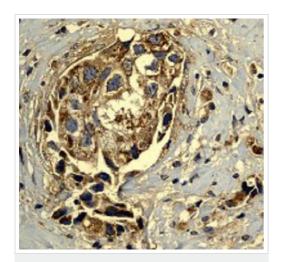
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hsp60 antibody (ab46798)

Immunohistochemical staining of paraffin embedded human cervical carcinoma with purified ab46798 at a working dilution of 1 in 100. The secondary antibody used is ab97051 Goat Anti-Rabbit IgG H&L (HRP) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



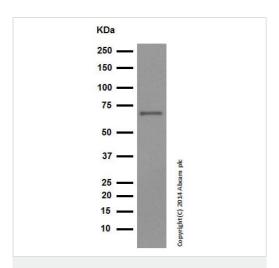
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hsp60 antibody (ab46798)

This image is courtesy of an Abreview submitted by Nicole Schechter Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/ Unpurified ab46798 staining Hsp60 from human colon tissue by immunohistochemistry (formalin/PFA-fixed paraffin-embedded sections). Cells were formaldehyde fixed prior to blocking in 10% serum for 2 hours at 21°C. The primary antibody was diluted 1/500 and incubated with the sample for 2 hours at 21°C. Alexa fluor® 594 goat polyclonal, diluted 1/5000, was used as the secondary.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Hsp60 antibody (ab46798)

Immunohistochemical analysis of paraffin-embedded human breast adenocarcinoma using unpurified ab46798 at 1/250 dilution.



Western blot - Anti-Hsp60 antibody (ab46798)

Anti-Hsp60 antibody (ab46798) at 1/50000 dilution (purified) + Pig heart tissue lysate at 20 μg

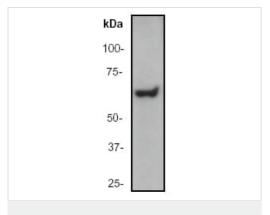
Secondary

HRP conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 60 kDa Observed band size: 60 kDa

Blocking Buffer: 5% NFDM/TBST

Dilution Buffer: 5% NFDM/TBST



Western blot - Anti-Hsp60 antibody (ab46798)

Anti-Hsp60 antibody (ab46798) at 1/50000 dilution (unpurified) + T-47D whole cell lysate (ab14899) at 10 μg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (<u>ab6721</u>) at 1/2000 dilution (Goat anti -rabbit HRP)

Predicted band size: 60 kDa Observed band size: 60 kDa

Secondary antibody - anti-rabbit HRP (ab6721)

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