abcam

Product datasheet

Anti-Hsp60 antibody [LK-1] ab59457

★★★★★ 3 Abreviews 16 References 画像数 7

製品の概要

製品名	Anti-Hsp60 antibody [LK-1]
製品の詳細	Mouse monoclonal [LK-1] to Hsp60
由来種	Mouse
アプリケーション	**適用あり:** WB, IP, Flow Cyt, IHC-P, ICC/IF
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Human Hsp60 produced through recombinant DNA methods in *E.coli*
ポジティブ・コントロール	HeLa cell lysate
特記事項	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

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
バッファー	Preservative: 0.09% Sodium azide Constituents: PBS, 50% Glycerol
精製度	Protein G purified
ポリ/モノ	モノクローナル
クローン名	LK-1
アイソタイプ	IgG1

アプリケーション

The Abpromise guarantee	Abpromise保証は、次のテスト済みアプリケーションにおけるab59457の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB	★★★★★ (2)	Use a concentration of 0.05 µg/ml. Predicted molecular weight: 60 kDa.
IP		Use a concentration of 5 µg/ml.
Flow Cyt		Use 1µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
IHC-P	★★★★☆ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. The antigen retrieval used on the human colon carcinoma IHC was 10% citric acid buffer, 121°C for 20 minutes.
ICC/IF		1/100.

ターゲット情報

機能

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.

関連疾患

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.

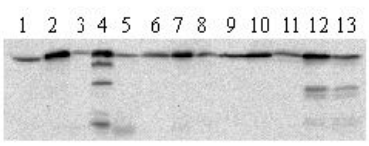
配列類似性

Belongs to the chaperonin (HSP60) family.

細胞内局在

Mitochondrion matrix.

画像



Western blot - Anti-Hsp60 antibody [LK-1] (ab59457)

All lanes : Anti-Hsp60 antibody [LK-1] (ab59457) at 0.05 µg/ml

Lane 1 : Rat Brain tissue lysates

Lane 2 : Rat Heart tissue lysates

Lane 3 : Rat Kidney tissue lysates

Lane 4 : Rat Liver tissue lysates

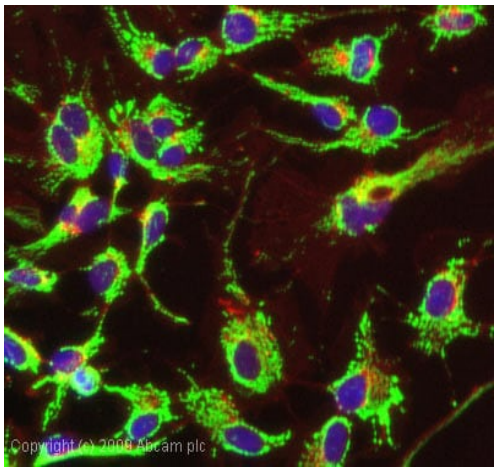
Lane 5 : Rat Lung tissue lysates

Lane 6 : Rat Pancreas tissue lysates
Lane 7 : Rat skeletal muscle tissue lysate
Lane 8 : Rat Spleen tissue lysate
Lane 9 : Rat Testes tissue lysate
Lane 10 : Rat Thymus tisuue lysate
Lane 11 : Cell lysates prepared from rat heart H9C2 cells
Lane 12 : Cell lysates prepared from mouse NIH3T3 cells
Lane 13 : Cell lysates prepared from mouse Pam212 cells

Lysates/proteins at 10 µg per lane.

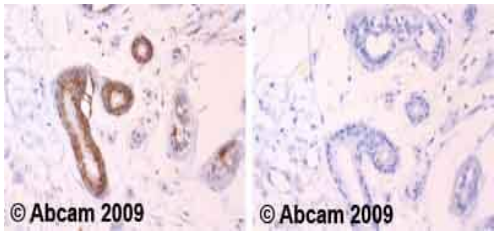
Secondary
All lanes : HRP-conjugated goat polyclonal to mouse IgG1 at 1/10 dilution

Predicted band size: 60 kDa



Immunocytochemistry/ Immunofluorescence - Anti-Hsp60 antibody [LK-1] (ab59457)

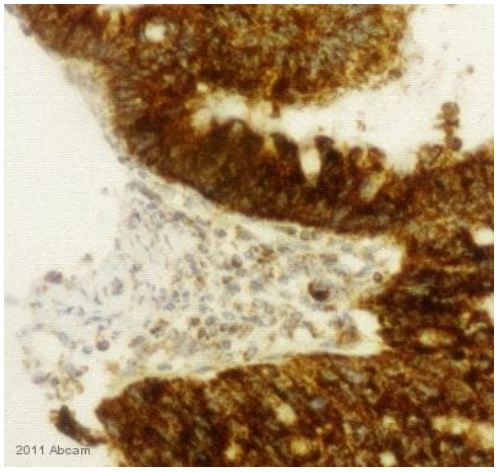
ICC/IF image of ab59457 stained HepG2 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab59457, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp60 antibody [LK-1] (ab59457)

Ab59457 staining human normal skin tissue. Staining is localised to mitochondria.
Left panel: with primary antibody at 2 ug/ml. Right panel: isotype control.
Sections were stained using an automated system DAKO Autostainer Plus , at room temperature. Sections were rehydrated and antigen retrieved with the Dako 3-in-1 antigen retrieval buffer EDTA pH 9.0 in a DAKO PT Link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 minutes and detected with Dako Envision Flex amplification kit for 30 minutes.

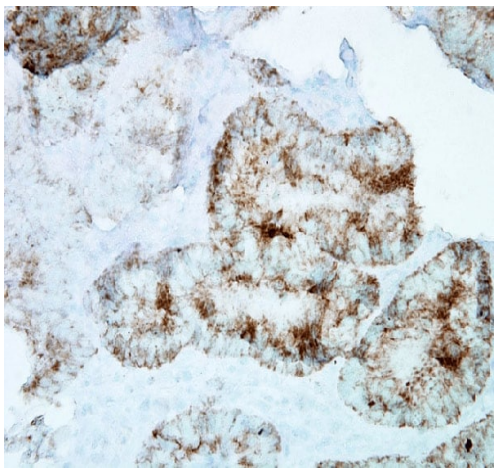
Colorimetric detection was completed with diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp60 antibody [LK-1] (ab59457)

This is image is courtesy of an Abreview submitted by Aamir Ahmed.

Ab59457 staining Hsp60 in Human malignant colon tissue sections by Immunohistochemistry. Antigen retrieval was by heat mediation using retrieval buffer, pH6. Cells were fixed with paraformaldehyde and blocked with 3% H_2O_2 for 10 minutes at 22°C. Samples were incubated with primary antibody at 1/2000 dilution for 2 hours at 22°C. A HRP conjugated goat polyclonal was used as a secondary antibody.



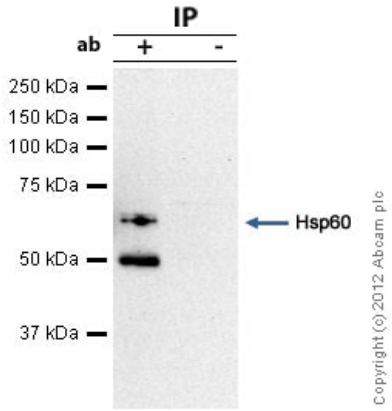
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp60 antibody [LK-1] (ab59457)

Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue labelling Hsp60 with ab59457 at 1/100,000 dilution for 1 hour at room temperature, followed by secondary antibody Goat Anti-Mouse (Biotin) at 1/2000 dilution for 1 hour at room temperature. Predominantly mitochondrial staining on Inflammatory cells in human colon carcinoma is observed. Counter stained with Hematoxylin.

Flow Cytometry - Anti-Hsp60 antibody [LK-1] (ab59457)

Overlay histogram showing HeLa cells stained with ab59457 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine

to block non-specific protein-protein interactions followed by the antibody (ab59457, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Immunoprecipitation - Anti-Hsp60 antibody [LK-1] (ab59457)

Hsp60 was immunoprecipitated using 0.5mg Rat Brain tissue lysate, 5µg of Mouse monoclonal to Hsp60 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, Rat Brain tissue lysate lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab59457.
Secondary: Goat polyclonal to mouse IgG light chain specific (HRP) at 1/20,000 dilution.
Band: 60kDa; Hsp60: non specific bands - 50kDa: We are unsure as to the identity of this extra band.

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