abcam

Product datasheet

Anti-ATP5A antibody [15H4C4] - Mitochondrial Marker ab14748

★★★★★ 25 Abreviews 482 References 画像数 9

製品の概要

製品名	Anti-ATP5A antibody [15H4C4] - Mitochondrial Marker
製品の詳細	Mouse monoclonal [15H4C4] to ATP5A - Mitochondrial Marker
由来種	Mouse
アプリケーション	**適用あり:** WB, IHC-P, ICC/IF, Flow Cyt
種交差性	**交差種:** Mouse, Rat, Cow, Human, Drosophila melanogaster **交差が予測される動物種:** Pig
免疫原	Tissue, cells or virus. This information is considered to be commercially sensitive.
ポジティブ・コントロール	WB: Isolated mitochondria from human, cow, rat and mouse heart. Human liver tissue lysate. HepG2 whole cell lysate. ICC/IF: HeLa, MCF7 and MDA-MB-231 cells. IHC-P: Human heart tissue. Flow Cyt: HepG2 cells.
特記事項	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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 +4°C.
バッファー	pH: 7.5 Preservative: 0.02% Sodium azide Constituent: HEPES buffered saline
精製度	IgG fraction
特記事項（精製）	Near homogeneity as judged by SDS-PAGE. The antibody was produced in vitro using hybridomas grown in serum-free medium, and then purified by biochemical fractionation.

ポリ/モノ	モノクローナル
クローン名	15H4C4
アイソタイプ	IgG2b
軽鎖の種類	kappa

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ab14748の使用に適用されます

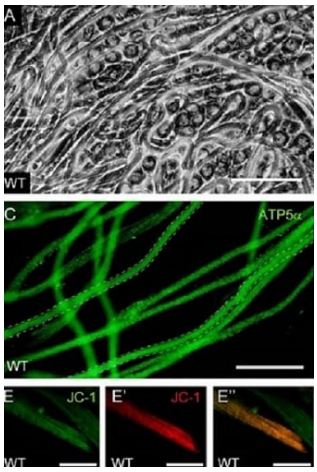
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB	★★★★★ (12)	Use a concentration of 1 µg/ml. Detects a band of approximately 53 kDa.
IHC-P	★★★★★ (6)	Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF	★★★★★ (3)	Use a concentration of 1 - 10 µg/ml.
Flow Cyt		Use a concentration of 1 µg/ml. **ab170192** - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.

ターゲット情報

機能	Mitochondrial membrane ATP synthase (F(1)F(0) ATP synthase or Complex V) produces ATP from ADP in the presence of a proton gradient across the membrane which is generated by electron transport complexes of the respiratory chain. F-type ATPases consist of two structural domains, F(1) - containing the extramembraneous catalytic core, and F(0) - containing the membrane proton channel, linked together by a central stalk and a peripheral stalk. During catalysis, ATP synthesis in the catalytic domain of F(1) is coupled via a rotary mechanism of the central stalk subunits to proton translocation. Subunits alpha and beta form the catalytic core in F(1). Rotation of the central stalk against the surrounding alpha(3)beta(3) subunits leads to hydrolysis of ATP in three separate catalytic sites on the beta subunits. Subunit alpha does not bear the catalytic high-affinity ATP-binding sites.
組織特異性	Fetal lung, heart, liver, gut and kidney. Expressed at higher levels in the fetal brain, retina and spinal cord.
配列類似性	Belongs to the ATPase alpha/beta chains family.
翻訳後修飾	The N-terminus is blocked.
細胞内局在	Mitochondrion inner membrane. Peripheral membrane protein.

画像



Immunocytochemistry/ Immunofluorescence - Anti-ATP5A antibody [15H4C4] - Mitochondrial Marker (ab14748)

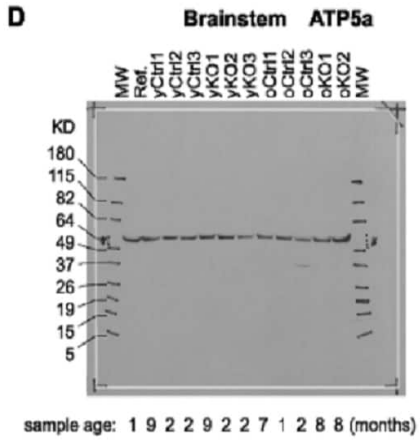
Vedelek et al PLoS One. 2016 Aug 16;11(8):e0161289. doi: 10.1371/journal.pone.0161289. eCollection 2016. Fig 4. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Testes of *bb8*^{ms} mutants show defects in post-meiotic, elongated spermatids.

(A-B) Spermatids from WT (A) and *bb8*^{ms} (B) testis both have elongated cysts, but there are large spherical vesicles in the mutant (arrows) by phase contrast microscopy. Scale bars: 100 μm.

(C, D) Mitochondria of elongated spermatids stained with ATP5α antibody ab14748 in WT (C) and in *bb8*^{ms} (D) mutants. ATP5α positive staining of the large vesicles in the cysts are indicated by arrow. Scale bars: 50 μm.

(E, F) JC-1 staining positive large vesicles (arrows) are absent from WT (E), but present in *bb8*^{ms} elongated cysts (F). Scale bars: 25 μm.



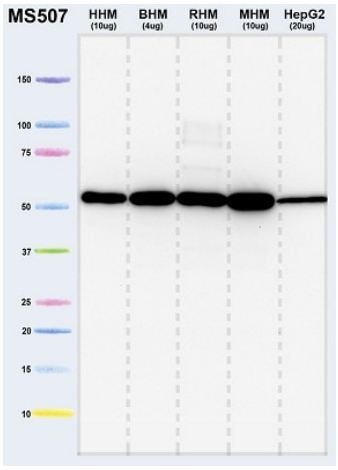
Western blot - Anti-ATP5A antibody [15H4C4] - Mitochondrial Marker (ab14748)

Kayser et al PLoS One. 2016 Jan 29;11(1):e0148219. doi: 10.1371/journal.pone.0148219. eCollection 2016. Fig S2. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Western blots were probed for HNE-damaged mitochondrial protein from brainstem (A), cerebellum (B) and “rest” brain (R). The sample designation indicates the age group (y for P25-P35, o for P45-P55), the genotype (KO, Ctrl for controls) and a number to distinguish independent samples.

Panel D is the blot from panel A reprobed for the mitochondrial marker ATPase (ATP5a) using ab14748 to demonstrate that extended sample storage did not degrade sample protein in general. Black lines in the MW lanes are magic marker on the film to indicate the positions of the prestained molecular weight standards on the blot.

For full image please see paper.



Western blot - Anti-ATP5A antibody [15H4C4] - Mitochondrial Marker (ab14748)

All lanes : Anti-ATP5A antibody [15H4C4] - Mitochondrial Marker (ab14748) at 1 µg/ml

Lane 1 : Isolated mitochondria from human heart at 10 µg
Lane 2 : Isolated mitochondria from bovine heart at 4 µg
Lane 3 : Isolated mitochondria from rat heart at 10 µg
Lane 4 : Isolated mitochondria from mouse heart at 10 µg
Lane 5 : HepG2 (Human liver hepatocellular carcinoma cell line) lysate at 20 µg

Western blot - Anti-ATP5A antibody [15H4C4] - Mitochondrial Marker (ab14748)

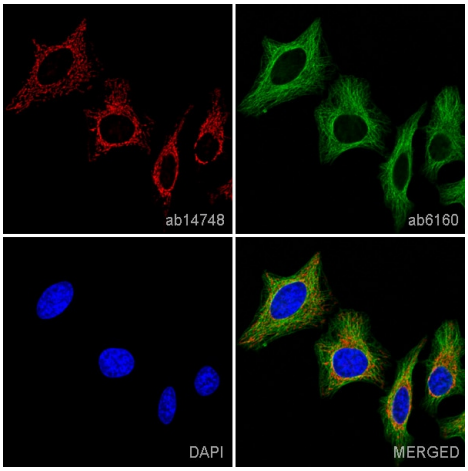
All lanes : Anti-ATP5A antibody [15H4C4] - Mitochondrial Marker (ab14748) at 1 µg/ml

Lane 1 : Human liver tissue lysate
Lane 2 : HepG2 (Human hepatocellular liver carcinoma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Observed band size: 55 kDa
Additional bands at: 36 kDa. We are unsure as to the identity of these extra bands.



Immunocytochemistry/ Immunofluorescence - Anti-ATP5A antibody [15H4C4] - Mitochondrial Marker (ab14748)

ICC/IF image of ab14748 stained HeLa (Human epithelial cell line from cervix adenocarcinoma) cells.

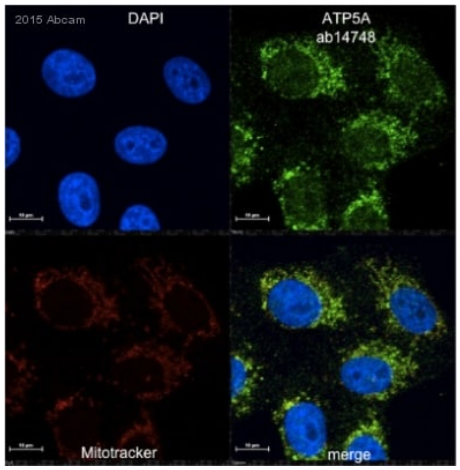
The cells were fixed in 100% methanol (5 minutes), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1 hour. The cells were then incubated overnight at +4°C with ab14748 at 1 µg/ml (shown in red) and **ab6160** (Rat monoclonal to Tubulin) at 1 µg/ml (shown in green).

This was followed by an incubation at room temperature for 1 hour with **ab150119**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 647) preadsorbed, at 0.5 µg/ml (shown in red) and **ab150165**, Goat Anti-Rat IgG H&L (Alexa Fluor® 488) preadsorbed, at 0.5 µg/ml (shown in green).

Nuclear DNA was labeled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This product also gave a positive signal under the same testing conditions in HeLa cells fixed with 4% formaldehyde (10 minutes).

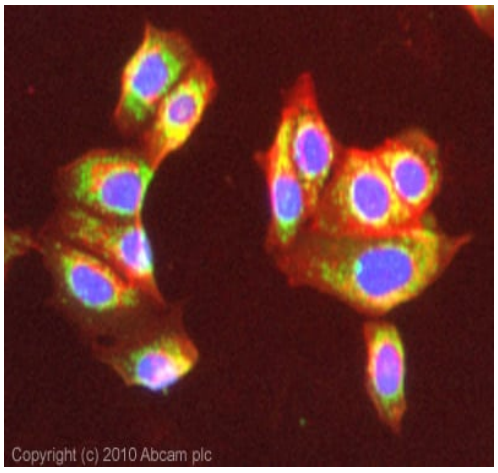


Immunocytochemistry/ Immunofluorescence - Anti-ATP5A antibody [15H4C4] - Mitochondrial Marker (ab14748)

This image is courtesy of an anonymous Abreview

ab14748 staining ATP5A in MDA-MB-231 cells by ICC/IF (Immunocytochemistry/immunofluorescence).

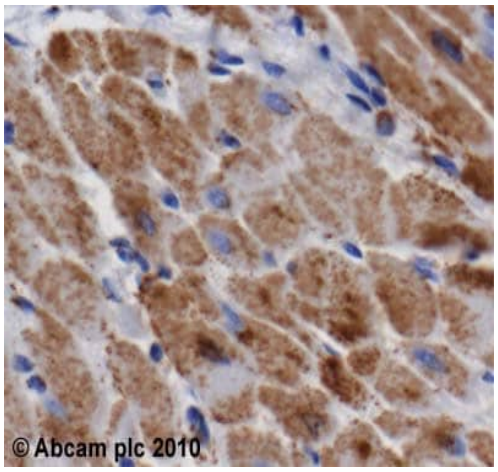
Cells were fixed with formaldehyde, permeabilized with 1% Triton X-100 and blocked with 10% BSA for 1 hour at 21°C. Samples were incubated with primary antibody (1/100 in BSA + 0.02% Tween 20) for 1 hour at 21°C. A DyLight® 550-conjugated goat anti-mouse IgG polyclonal (1/500) was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-ATP5A antibody [15H4C4] - Mitochondrial Marker (ab14748)

ICC/IF image of ab14748 stained MCF7 (Human breast adenocarcinoma cell line) cells.

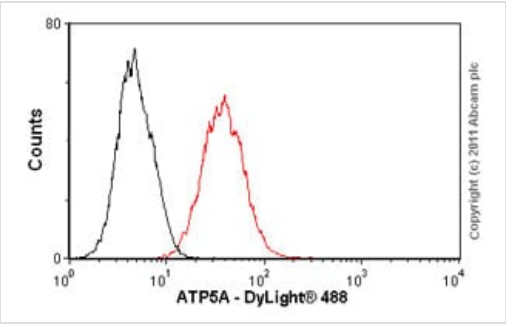
The cells were fixed in 4% formaldehyde (10 minutes) and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1 hour to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab14748, 10 µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1 hour. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1 hour. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43 µM.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATP5A antibody [15H4C4] - Mitochondrial Marker (ab14748)

ab14748 (1 µg/ml) staining ATP5A in human heart (left ventricle), using an automated system (DAKO Autostainer Plus). Using this protocol there is mitochondrial staining of cardiomyocytes. Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer citrate pH 6.1 in a DAKO PT link. Slides were peroxidase blocked in 3% H_2O_2 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 hematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.



Flow Cytometry - Anti-ATP5A antibody [15H4C4] - Mitochondrial Marker (ab14748)

Overlay histogram showing HepG2 (Human liver hepatocellular carcinoma cell line) cells stained with ab14748 (red line).

The cells were fixed with 4% paraformaldehyde (10 minutes) and then permeabilized with 0.1% PBS-Tween for 20 minutes. 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 (ab14748, 1 µg/1x10^6 cells) for 30 minutes at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 minutes at 22ºC. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (**ab91366**, 2 µg/1x10^6 cells) used under the same conditions.

Acquisition of >5,000 events was performed.

This antibody gave a positive signal in HepG2 cells fixed with 80% methanol (5 minutes)/permeabilized in 0.1% PBS-Tween used under the same conditions.