abcam

ab200652

Anti-HADHA antibody [EPR17939]

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR17939
Purification technique	Affinity purification Protein A
Concentration	0.327 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/250
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1 µg/mL
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information HADHA

Function

Mitochondrial trifunctional enzyme catalyzes the last three of the four reactions of the mitochondrial beta-oxidation pathway (PubMed:1550553, PubMed:29915090, PubMed:30850536, PubMed:8135828, PubMed:31604922). The mitochondrial beta-oxidation pathway is the major energy-producing process in tissues and is performed through four consecutive reactions breaking down fatty acids into acetyl-CoA (PubMed:29915090). Among the enzymes involved in this pathway, the trifunctional enzyme exhibits specificity for long-chain fatty acids (PubMed:30850536, PubMed:31604922). Mitochondrial trifunctional enzyme is a heterotetrameric complex composed of two proteins, the trifunctional enzyme subunit alpha/HADHA described here carries the 2,3-enoyl-CoA hydratase and the 3-hydroxyacyl-CoA dehydrogenase activities while the trifunctional enzyme subunit beta/HADHB bears the 3-ketoacyl-CoA thiolase activity (PubMed:29915090, PubMed:30850536, PubMed:8135828). Independently of subunit beta, HADHA also exhibits a cardiolipin acyltransferase activity that participates in cardiolipin remodeling; cardiolipin is a major mitochondrial membrane phospholipid (PubMed:23152787, PubMed:31604922). HADHA may act downstream of Tafazzin/TAZ, that remodels monolysocardiolipin (MLCL) to a cardiolipin intermediate, and then HADHA may continue to remodel this species into mature tetralinoleoyl-cardiolipin (PubMed:31604922). Has also been proposed to act directly on MLCL; capable of acylating MLCL using different acyl-CoA substrates, with highest activity for oleoyl-CoA (PubMed:23152787).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks

Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The HADHA protein also called hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha is an essential component of the mitochondrial trifunctional protein complex. This protein has a molecular mass of about 79 kDa and is expressed mainly in tissues with high fatty acid oxidation rates like liver heart and muscle. HADHA plays a significant role in the beta-oxidation of long-chain fatty acids acting on hydroxyacyl-CoA substrates during this critical metabolic process.
Biological function summary	HADHA is a part of the mitochondrial trifunctional protein complex which consists of four alpha and four beta subunits. It facilitates the hydration of enoyl-CoA to 3-hydroxyacyl-CoA and the subsequent dehydrogenation to 3-ketoacyl-CoA. This enzyme works closely with its partner the HADHB protein to carry out these reactions efficiently. These functions are important for energy production as they are steps in the breakdown of fatty acids necessary for ATP generation.
Pathways	HADHA participates in the mitochondrial beta-oxidation pathway an essential pathway for energy production from fats. Alongside HADHB it catalyzes key reactions that allow the progressive shortening of fatty acid chains which further feeds into the citric acid cycle. This pathway links HADHA not only to HADHB but

	also to other enzymes involved in lipid metabolism and energy homeostasis including medium-chain specific acyl-CoA dehydrogenase (MCAD) reflecting its role in comprehensive metabolic networks.
Associated diseases and disorders	Defects in HADHA are associated with mitochondrial trifunctional protein deficiency and long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. Both disorders disrupt normal fatty acid oxidation leading to a spectrum of symptoms including hypoketotic hypoglycemia and cardiomyopathy. These conditions highlight the relationship between HADHA and other proteins involved in fatty acid metabolism such as HADHB further highlighting their collective role in maintaining cellular energy balance.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

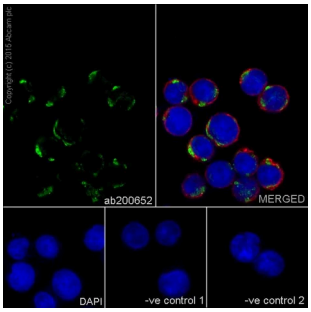
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

10 product images



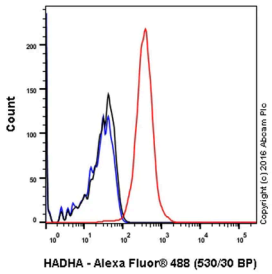
Immunocytochemistry/ Immunofluorescence - Anti-HADHA antibody [EPR17939] (ab200652)

HADHA Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HADHA antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling HADHA with ab200652 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green).
Cytoplasm staining on Jurkat cell line is observed.

The nuclear counter stain is DAPI (blue).

Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).
The negative controls are as follows:
-ve control 1: ab200652 at 1/250 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.

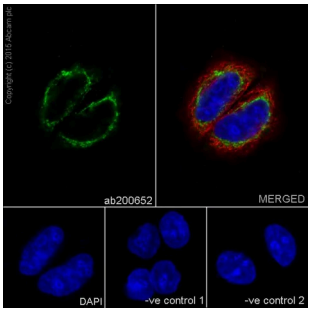


Flow Cytometry (Intracellular) - Anti-HADHA antibody [EPR17939] (ab200652)

HADHA Flow Cytometry (Intracellular) staining using rabbit Anti-HADHA antibody

ab200652 staining HADHA in Jurkat (human acute T cell leukemia) cellsby intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/2200. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.
Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



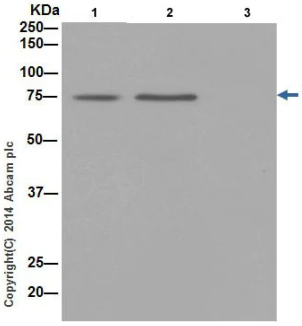
Immunocytochemistry/ Immunofluorescence - Anti-HADHA antibody [EPR17939] (ab200652)

HADHA Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HADHA antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling HADHA with ab200652 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green).
Cytoplasm staining on HeLa cell line is observed.

The nuclear counter stain is DAPI (blue).

Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).
The negative controls are as follows:
-ve control 1: ab200652 at 1/250 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.



Immunoprecipitation - Anti-HADHA antibody [EPR17939] (ab200652)

HADHA was immunoprecipitated from 1mg of HEK293 (Human embryonic kidney) whole cell lysate with ab200652 at 1/30 dilution.
Western blot was performed from the immunoprecipitate using ab200652 at 1/2000 dilution.

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: HEK293 whole cell lysate 10 µg (Input).

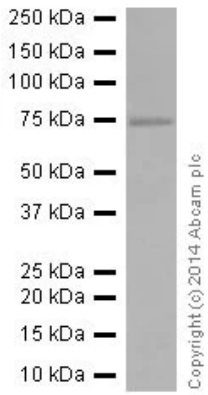
Lane 2: ab200652 IP in HEK293 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab200652 in HEK293 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-HADHA antibody [EPR17939] (ab200652)

Predicted band size: 83 kDa



Western blot - Anti-HADHA antibody [EPR17939] (ab200652)

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-HADHA antibody [EPR17939] (ab200652) at 1/10000 dilution

All lanes:
HepG2 (Human liver hepatocellular carcinoma) whole cell lysate at 20 µg

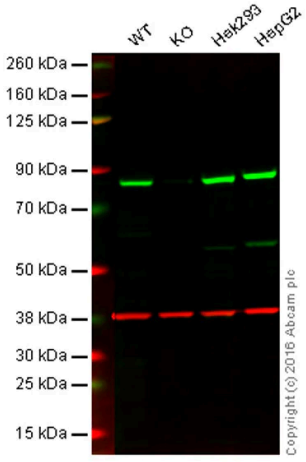
Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 62 kDa, 66 kDa, 83 kDa

Observed band size: 62 kDa, 74 kDa

Exposure time: 3min



Western blot - Anti-HADHA antibody [EPR17939] (ab200652)

HADHA Western blot staining using rabbit Anti-HADHA antibody

Lanes 1 - 4: Merged signal (red and green). Green - ab200652 observed at 82 kDa. Red - loading control, ab8245, observed at 37 kDa.
ab200652 was shown to specifically react with HADHA when HADHA knockout samples were used. Wild-type and HADHA knockout samples were subjected to SDS-PAGE. ab200652 and ab8245 (loading control to GAPDH) were diluted at 1/1000 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed ab216776 secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.
All lanes:
Western blot - Anti-HADHA antibody [EPR17939] (ab200652) at 1/1000 dilution

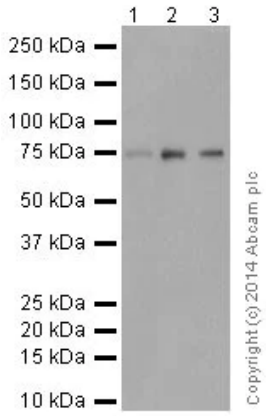
Lane 1:
Wild-type HAP1 cell lysate at 20 µg

Lane 2:
HADHA knockout HAP1 cell lysate at 20 µg

Lane 3:
HEK293 cell lysate at 20 µg

Lane 4:
HepG2 cell lysate at 20 µg

Predicted band size: 83 kDa



Western blot - Anti-HADHA antibody [EPR17939] (ab200652)

HADHA Western blot staining using rabbit Anti-HADHA antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-HADHA antibody [EPR17939] (ab200652) at 1/1000 dilution

Lane 1:
Human fetal brain lysate at 10 µg

Lane 2:
Human fetal kidney lysate at 10 µg

Lane 3:
Human fetal liver lysate at 10 µg

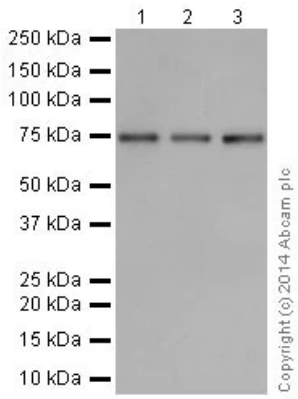
Secondary

All lanes:
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 83 kDa

Observed band size: 74 kDa

Exposure time: 1s



Western blot - Anti-HADHA antibody [EPR17939] (ab200652)

HADHA Western blot staining using rabbit Anti-HADHA antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-HADHA antibody [EPR17939] (ab200652) at 1/1000 dilution

Lane 1:
HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate at 20 µg

Lane 2:
Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate at 20 µg

Lane 3:
HEK293 (Human embryonic kidney) whole cell lysate at 20 µg

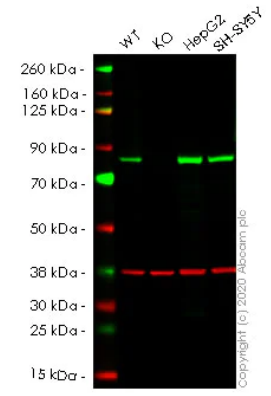
Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 83 kDa

Observed band size: 74 kDa

Exposure time: 1s



Western blot - Anti-HADHA antibody [EPR17939] (ab200652)

HADHA Western blot staining using rabbit Anti-HADHA antibody

Lanes 1-4: Merged signal (red and green). Green - ab200652 observed at 82 kDa. Red - loading control ab8245 observed at 37 kDa.
ab200652 Anti-HADHA antibody [EPR17939] was shown to specifically react with HADHA in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266274 (knockout cell lysate ab257464) was used. Wild-type and HADHA knockout samples were subjected to SDS-PAGE. ab200652 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-HADHA antibody [EPR17939] (ab200652) at 1/1000 dilution

Lane 1:
Wild-type HEK-293T cell lysate at 20 µg

Lane 2:
Western blot - Human HADHA knockout HEK-293T cell lysate (ab257464) at 20 µg

Lane 3:

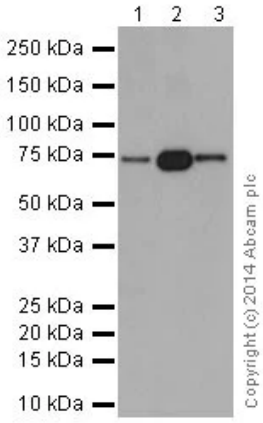
HepG2 cell lysate at 20 µg

Lane 4:
SH-SY5Y cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 83 kDa

Observed band size: 82 kDa



Western blot - Anti-HADHA antibody [EPR17939] (ab200652)

HADHA Western blot staining using rabbit Anti-HADHA antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-HADHA antibody [EPR17939] (ab200652) at 1/1000 dilution

Lane 1:
Mouse kidney lysate at 10 µg

Lane 2:
Rat heart lysate at 10 µg

Lane 3:
Rat kidney lysate at 10 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 83 kDa

Observed band size: 74 kDa

Exposure time: 3min

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.