abcam

Product datasheet

Anti-HADHA antibody [EPR17939] - BSA and Azide free ab242411

KO VALIDATED Recombinant RabMAb

7 Images

Overview

Product name	Anti-HADHA antibody [EPR17939] - BSA and Azide free
Description	Rabbit monoclonal [EPR17939] to HADHA - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IP, Flow Cyt, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human HADHA aa 250-350. The exact sequence is proprietary. Database link: P40939
Positive control	WB: HeLa, Jurkat, HEK293, SH-SY5Y and HepG2 whole cell lysates; Human fetal brain, fetal kidney and fetal liver lysates; Mouse kidney, rat heart and rat kidney lysates. ICC/IF: Jurkat and HeLa cells. IP: HEK293 whole cell lysate. Flow Cyt: Jurkat (human acute T cell leukemia).
General notes	Ab242411 is the carrier-free version of ab200652. This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. ab242411 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *Maxpar® is a trademark of Fluidigm Canada Inc.* 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 production For more information see here. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents. Reproducibility is key to advancing scientific discovery and accelerating scientists' next

breakthrough.

Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.

We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee.

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

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, as well as customer reviews and Q&As.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR17939
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab242411** in the following tested applications.

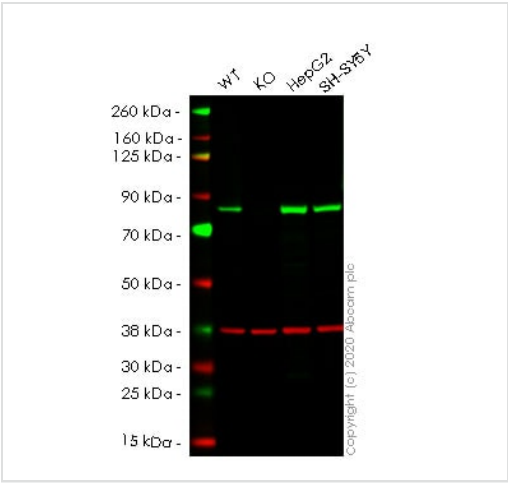
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 74 kDa (predicted molecular weight: 83 kDa).

Target

Function	Bifunctional subunit.
Pathway	Lipid metabolism; fatty acid beta-oxidation.
Involvement in disease	Defects in HADHA are a cause of trifunctional protein deficiency (TFP deficiency) [MIM:609015]. The clinical manifestations are very variable and include hypoglycemia, cardiomyopathy and sudden death. Phenotypes with mainly hepatic and neuromyopathic involvement can also be distinguished. Biochemically, TFP deficiency is defined by the loss of all enzyme activities of the TFP complex. Defects in HADHA are the cause of long-chain 3-hydroxyl-CoA dehydrogenase deficiency (LCHAD deficiency) [MIM:609016]. The clinical features are very similar to TFP deficiency. Biochemically, LCHAD deficiency is characterized by reduced long-chain 3-hydroxyl-CoA dehydrogenase activity, while the other enzyme activities of the TFP complex are normal or only slightly reduced. Defects in HADHA are a cause of maternal acute fatty liver of pregnancy (AFLP) [MIM:609016]. AFLP is a severe maternal illness occurring during pregnancies with affected fetuses. This disease is associated with LCHAD deficiency and characterized by sudden unexplained infant death or hypoglycemia and abnormal liver enzymes (Reye-like syndrome).
Sequence similarities	In the N-terminal section; belongs to the enoyl-CoA hydratase/isomerase family. In the central section; belongs to the 3-hydroxyacyl-CoA dehydrogenase family.
Cellular localization	Mitochondrion.

Images



Western blot - Anti-HADHA antibody [EPR17939] - BSA and Azide free (ab242411)

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

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : H2AFY Knockout HEK-293T cell lysate
Lane 3 : HepG2 cell lysate
Lane 4 : SH-SY5Y cell lysate

Lysates/proteins at 20 µg per lane.

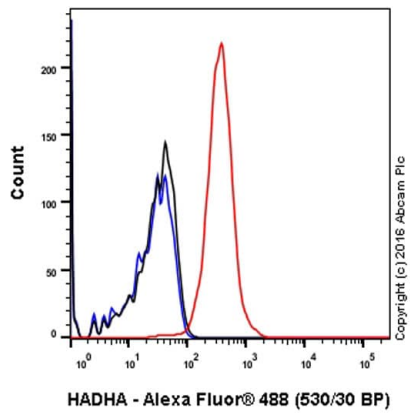
Performed under reducing conditions.

Predicted band size: 83 kDa
Observed band size: 82 kDa
why is the actual band size different from the predicted?

This data was developed using the same antibody clone in a different buffer formulation (ab200652).

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.



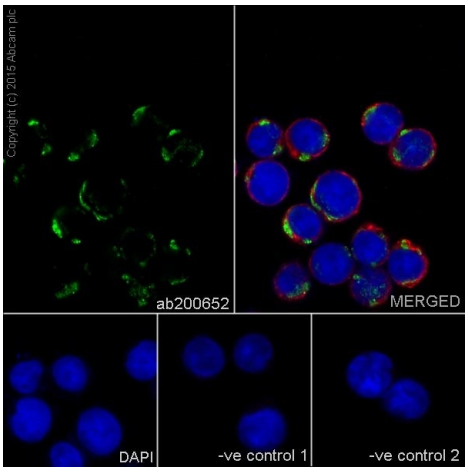
Flow Cytometry - Anti-HADHA antibody [EPR17939] - BSA and Azide free (ab242411)

ab200652 staining HADHA in Jurkat (human acute T cell leukemia) cells by 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)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab200652)



Immunocytochemistry/ Immunofluorescence - Anti-HADHA antibody [EPR17939] - BSA and Azide free (ab242411)

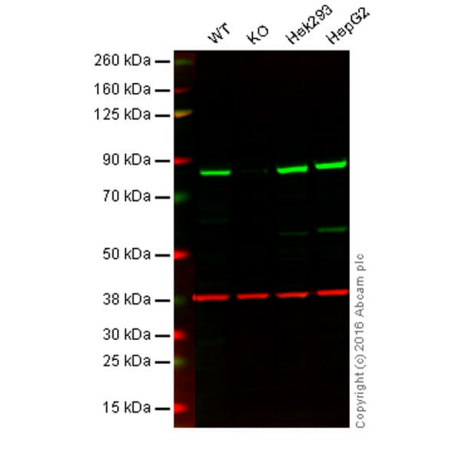
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling HADHA with ab200652 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab200652)



Western blot - Anti-HADHA antibody [EPR17939] - BSA and Azide free (ab242411)

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

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : HADHA knockout HAP1 cell lysate
Lane 3 : HEK293 cell lysate
Lane 4 : HepG2 cell lysate

Lysates/proteins at 20 µg per lane.

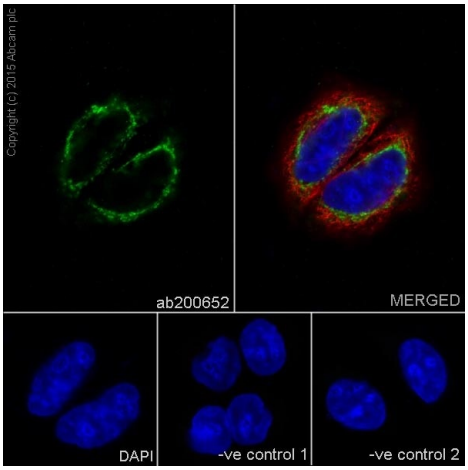
Predicted band size: 83 kDa

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab200652)



Immunocytochemistry/ Immunofluorescence - Anti-HADHA antibody [EPR17939] - BSA and Azide free (ab242411)

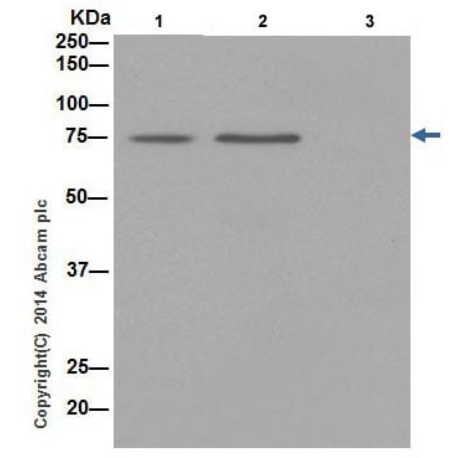
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling HADHA with ab200652 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green).

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab200652)



Immunoprecipitation - Anti-HADHA antibody [EPR17939] - BSA and Azide free (ab242411)

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).

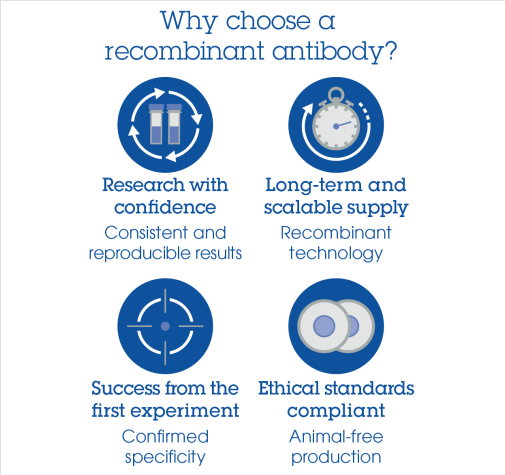
Lane 2: ab200652 IP in HEK293 whole cell lysate.

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

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and

sodium azide (ab200652)



Anti-HADHA antibody [EPR17939] - BSA and Azide free (ab242411)

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