abcam

ab203114

Anti-HADHA antibody [EPR17940]

Recombinant | RabMAb | KO Validated | 20ul selling size

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	EPR17940
Purification technique	Affinity purification Protein A
Concentration	0.596 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/40
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 µg/mL
Notes	-

Expected

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

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

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

IHC-P

Tested

Species	Human
Dilution info	1/100
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

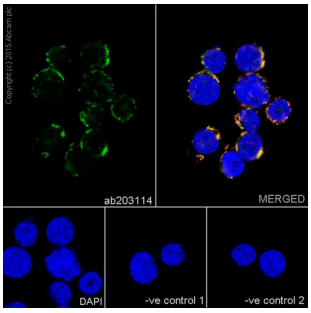
We do not recommend this combination. It is not covered by our product promise.

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.

14 product images

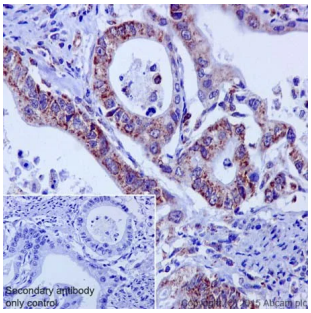


Immunocytochemistry/ Immunofluorescence - Anti-HADHA antibody [EPR17940] (ab203114)

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 ab203114 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on Jurkat cell line.
The nuclear counter stain is DAPI (blue). COX IV is detected with ab33985 (anti-COX IV Mitochondrial Marker mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:
-ve control 1: ab203114 at 1/250 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.
-ve control 2: ab33985 (anti-COX IV Mitochondrial Marker mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.

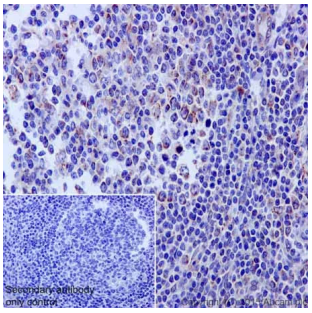


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HADHA antibody [EPR17940] (ab203114)

HADHA Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HADHA antibody

Immunohistochemical analysis of paraffin-embedded Human cervix carcinoma tissue labeling HADHA with ab203114 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic staining on Human cervix carcinoma tissue is observed. Counter stained with Hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

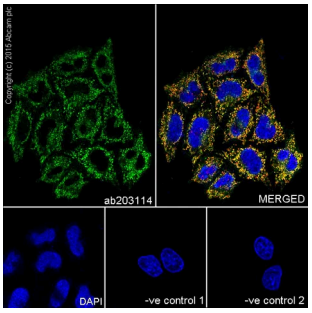


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HADHA antibody [EPR17940] (ab203114)

HADHA Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HADHA antibody

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling HADHA with ab203114 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic staining on Human tonsil tissue is observed. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

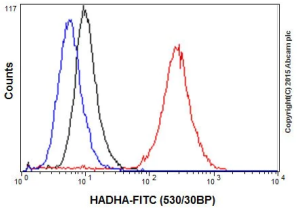


Immunocytochemistry/ Immunofluorescence - Anti-HADHA antibody [EPR17940] (ab203114)

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 ab203114 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on HeLa cell line.
The nuclear counter stain is DAPI (blue). COX IV is detected with ab33985 (anti-COX IV Mitochondrial Marker mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

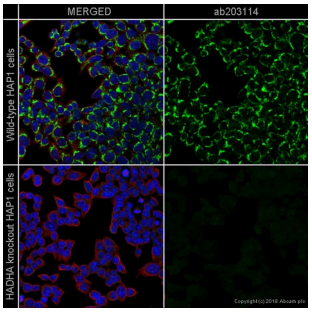
The negative controls are as follows:
-ve control 1: ab203114 at 1/250 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.
-ve control 2: ab33985 (anti-COX IV Mitochondrial Marker mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-HADHA antibody [EPR17940] (ab203114)

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

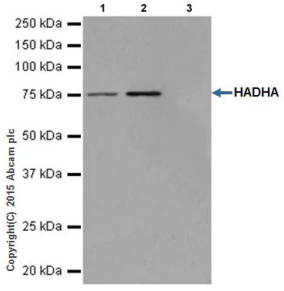
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling HADHA with ab203114 at 1/100 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-HADHA antibody [EPR17940] (ab203114)

HADHA Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HADHA antibody

ab203114 staining HADHA in wild-type HAP1 cells (top panel) and HADHA knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol for 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 with ab203114 at 1µg/ml concentration dilution and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1 hour with Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) secondary antibody at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunoprecipitation - Anti-HADHA antibody [EPR17940] (ab203114)

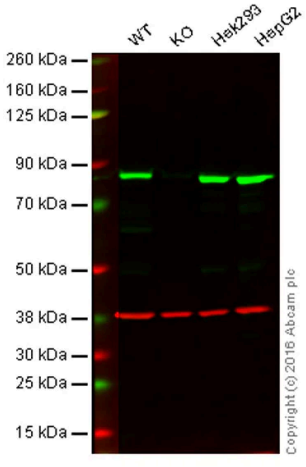
HADHA was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate with ab203114 at 1/400 dilution. Western blot was performed from the immunoprecipitate using ab203114 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.
Lane 1: HeLa whole cell lysate 10ug (Input). Lane 2: ab203114 IP in HeLa whole cell lysate. Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab203114 in HeLa whole cell lysate.

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

Exposure time: 3 seconds.

All lanes:
Immunoprecipitation - Anti-HADHA antibody [EPR17940] (ab203114)

Predicted band size: 83 kDa



Western blot - Anti-HADHA antibody [EPR17940] (ab203114)

HADHA Western blot staining using rabbit Anti-HADHA antibody

Lanes 1 - 4: Merged signal (red and green). Green - ab203114 observed at 82 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab203114 was shown to specifically react with HADHA when HADHA knockout samples were used. Wild-type and HADHA knockout samples were subjected to SDS-PAGE. ab203114 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 1/10000 dilution for 1 h at room temperature before imaging.
All lanes:
Western blot - Anti-HADHA antibody [EPR17940] (ab203114) at 1/1000 dilution

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

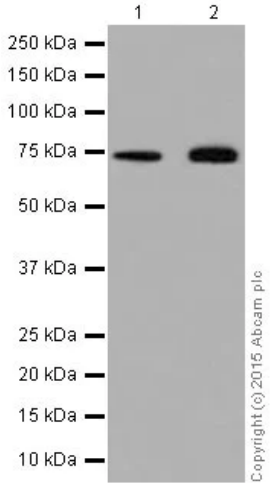
Lane 2:
HADHA knockout HAP1 cell lysate at 20 µg

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 [EPR17940] (ab203114)

HADHA Western blot staining using rabbit Anti-HADHA antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-HADHA antibody [EPR17940] (ab203114) at 1/1000 dilution

Lane 1:
Human fetal liver lysate at 10 µg

Lane 2:
Human fetal kidney lysate at 10 µg

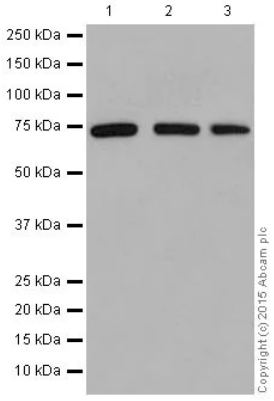
Secondary

All lanes:
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/50000 dilution

Predicted band size: 83 kDa

Observed band size: 74 kDa

Exposure time: 30s



Western blot - Anti-HADHA antibody [EPR17940] (ab203114)

HADHA Western blot staining using rabbit Anti-HADHA antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-HADHA antibody [EPR17940] (ab203114) at 1/10000 dilution

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

Lane 2:
HEK-293 (Human epithelial cells from embryonic kidney) cell lysate at 20 µg

Lane 3:
HepG2 (Human liver hepatocellular carcinoma) cell lysate at 20 µg

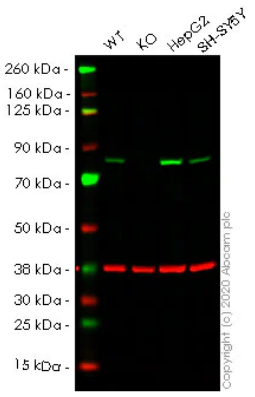
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 83 kDa

Observed band size: 74 kDa

Exposure time: 3min



Western blot - Anti-HADHA antibody [EPR17940] (ab203114)

HADHA Western blot staining using rabbit Anti-HADHA antibody

Lanes 1-4: Merged signal (red and green). Green - ab203114 observed at 82 kDa. Red - loading control ab8245 observed at 37 kDa.
ab203114 Anti-HADHA antibody [EPR17940] 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. ab203114 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 [EPR17940] (ab203114) at 1/1000 dilution

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

Lane 2:
HADHA knockout HEK-293T cell lysate at 20 µg

Lane 2:
Western blot - Human HADHA knockout HEK-293T cell line (ab266274)

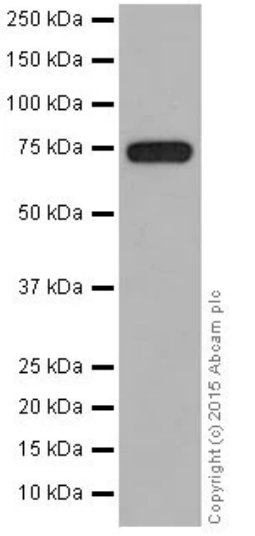
Lane 3:
HepG2 cell lysate at 20 µg

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 [EPR17940] (ab203114)

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-HADHA antibody [EPR17940] (ab203114) at 1/1000 dilution

All lanes:
Jurkat (Human T cell leukemia cells from peripheral blood) cell lysate at 20 µg

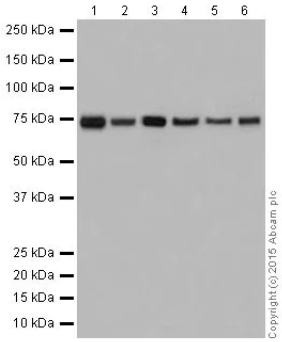
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 83 kDa

Observed band size: 74 kDa

Exposure time: 3min



Western blot - Anti-HADHA antibody [EPR17940] (ab203114)

HADHA Western blot staining using rabbit Anti-HADHA antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-HADHA antibody [EPR17940] (ab203114) at 1/2000 dilution

Lane 1:
Mouse heart lysate at 10 µg

Lane 2:
Mouse kidney lysate at 10 µg

Lane 3:
Rat heart lysate at 10 µg

Lane 4:
Rat kidney lysate at 10 µg

Lane 5:
C6 (Rat glial tumor cells) cell lysate at 10 µg

Lane 6:
RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) cell lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 83 kDa

Observed band size: 74 kDa

Exposure time: 15s

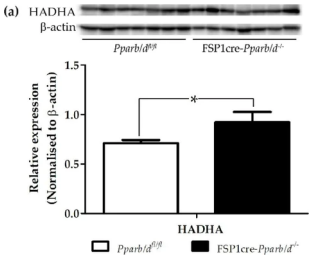


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Western blot - Anti-HADHA antibody [EPR17940] (ab203114)

HADHA Western blot staining using rabbit Anti-HADHA antibody

HADHA western blot using anti-HADHA antibody [EPR17940] ab203114. Publication image and figure legend from Chen, J., Zhuang, Y., et al., 2019, Int J Mol Sci, PubMed 31618976.

ab203114 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab203114 please see the product overview.

Expression levels of proteins in fatty acid β-oxidation, fatty acid synthesis, and triglyceride synthesis pathways. Immunoblot analysis of indicated proteins in the liver of P2 Pparb/dfl/fl and FSP1cre-Pparb/d-/- mice. Representative blots from 7 mice (biological replicates) for each genotype and the results of the 6-7 mice (biological replicates) are shown in bar graphs. Two-tailed Mann-Whitney test with values shown as mean ± s.e.m. * $p < 0.05$, ** $p < 0.01$; FSP1cre-Pparb/d-/- vs. Pparb/dfl/fl controls. (a) HADHA. β-actin was used as loading and transfer control. (b) Phospho-ACLY and ACLY. β-tubulin was used as loading and transfer control. (c) Phospho-ACC and ACC. β-

tubulin was used as loading and transfer control. (d) GPD2. β-actin was used as loading and transfer control. The full size original western blots are shown in Figure SM2.

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