abcam

Product datasheet

Anti-HADHA antibody [EPR17940] ab203114

KO VALIDATED Recombinant RabMAb

7 References 14 Images

Overview

Product name	Anti-HADHA antibody [EPR17940]
Description	Rabbit monoclonal [EPR17940] to HADHA
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IP, IHC-P, WB, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, HEK-293, HepG2, Jurkat, C6 and RAW 264.7 cell lysates; Human fetal liver and fetal kidney lysates; Mouse heart and kidney lysates; Rat heart and kidney lysates. IHC-P: Human cervix carcinoma and tonsil tissues. ICC/IF: Jurkat, HeLa cells, HAP1 wildtype and HAP1-HADHA knockout cells Flow Cyt (intra): HeLa cells. IP: HeLa whole cell lysate.
General notes	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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR17940

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab203114 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 a concentration of 1 µg/ml.
IP		1/40.
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 74 kDa (predicted molecular weight: 83 kDa).
Flow Cyt (Intra)		1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

Function Bifunctional subunit.

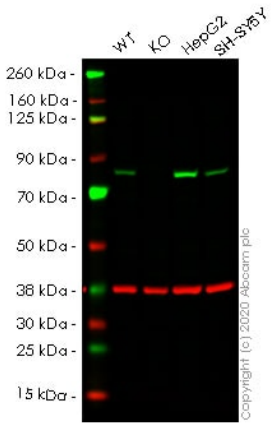
Pathway Lipid metabolism; fatty acid beta-oxidation.

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 [EPR17940] (ab203114)

All lanes : Anti-HADHA antibody [EPR17940] (ab203114) at 1/1000 dilution

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

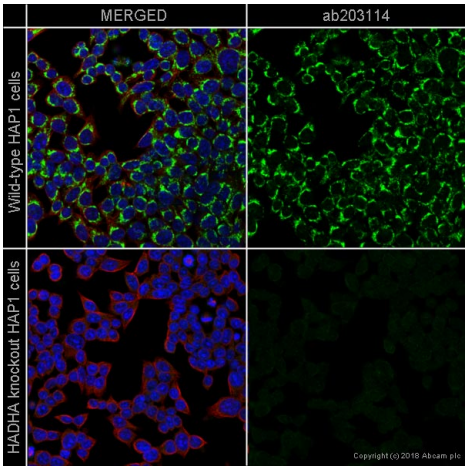
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 83 kDa
Observed band size: 82 kDa

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

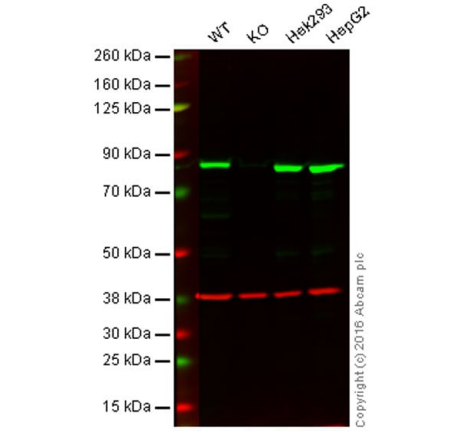
ab203114 Anti-HADHA antibody [EPR17940] was shown to specifically react with HADHA in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line **ab266274** (knockout cell lysate **ab257464**) was used. Wild-type and HADHA knockout samples were subjected to SDS-PAGE. ab203114 and 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.



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

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).



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

All lanes : Anti-HADHA antibody [EPR17940] (ab203114) at 1/1000 dilution

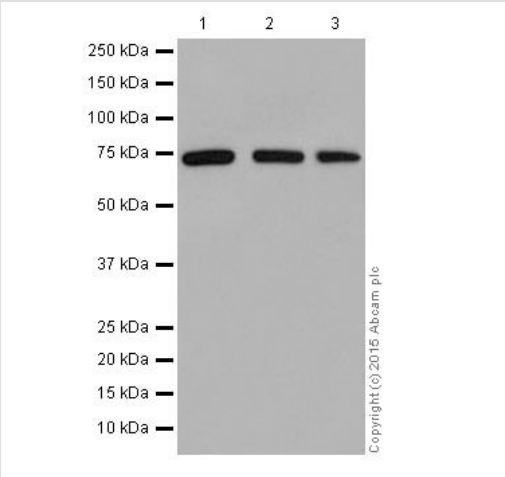
Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : HADHA knockout HAP1 cell lysate
Lane 3 : HEK293 cell lysate
Lane 4 : HepG2 cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 83 kDa

Lanes 1 - 4: Merged signal (red and green). Green - 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.



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

All lanes : Anti-HADHA antibody [EPR17940] (ab203114) at 1/10000 dilution

Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) cell lysate
Lane 2 : HEK-293 (Human epithelial cells from embryonic kidney) cell lysate
Lane 3 : HepG2 (Human liver hepatocellular carcinoma) cell lysate

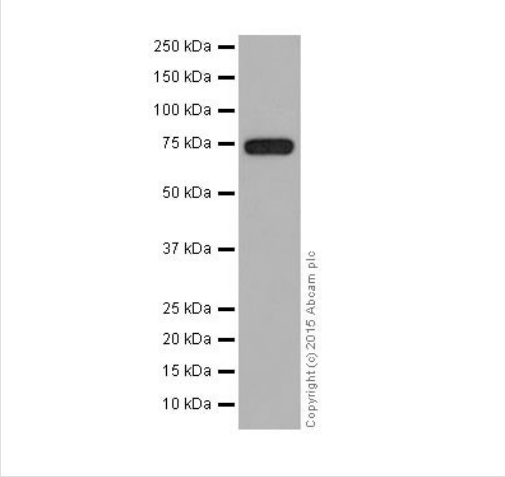
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 83 kDa
Observed band size: 74 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



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

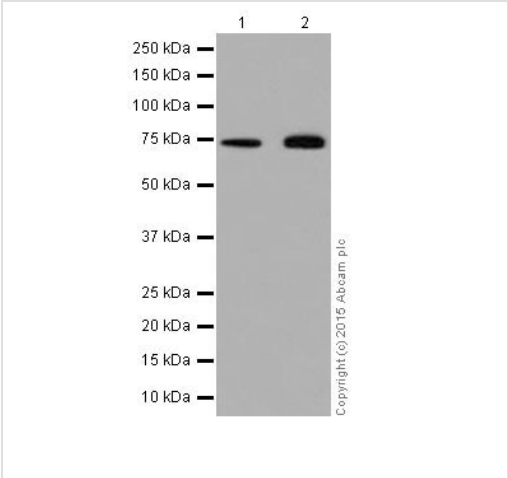
Anti-HADHA antibody [EPR17940] (ab203114) at 1/1000 dilution + Jurkat (Human T cell leukemia cells from peripheral blood) cell lysate at 20 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 83 kDa
Observed band size: 74 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



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

All lanes : Anti-HADHA antibody [EPR17940] (ab203114) at 1/1000 dilution

Lane 1 : Human fetal liver lysate
Lane 2 : Human fetal kidney lysate

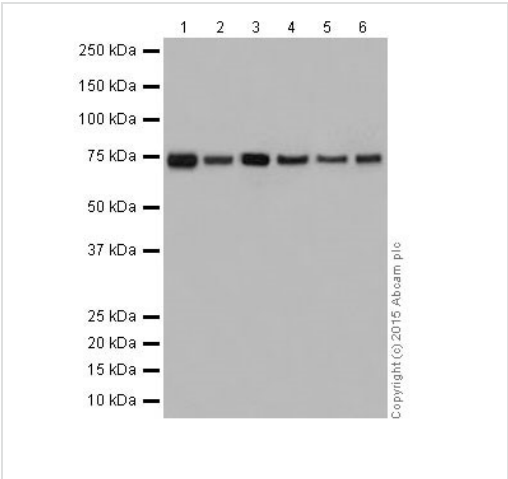
Lysates/proteins at 10 µg per lane.

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: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



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

All lanes : Anti-HADHA antibody [EPR17940] (ab203114) at 1/2000 dilution

Lane 1 : Mouse heart lysate
Lane 2 : Mouse kidney lysate
Lane 3 : Rat heart lysate
Lane 4 : Rat kidney lysate
Lane 5 : C6 (Rat glial tumor cells) cell lysate
Lane 6 : RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) cell lysate

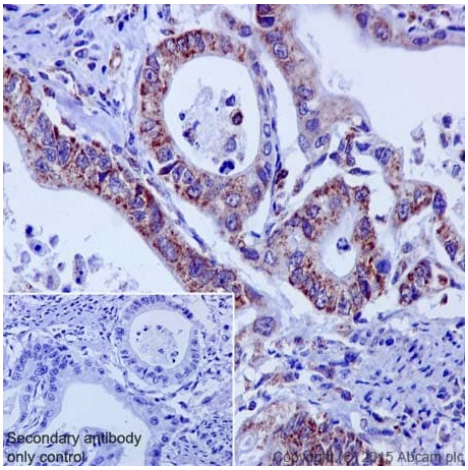
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 83 kDa
Observed band size: 74 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

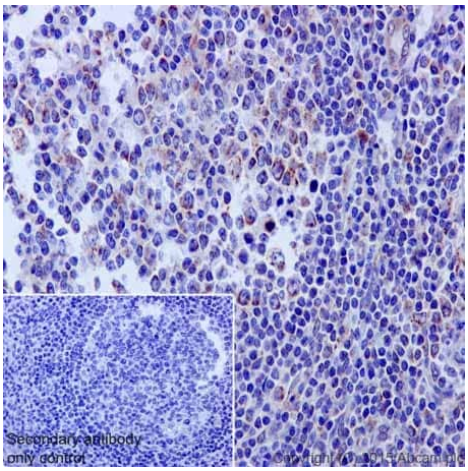


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HADHA antibody [EPR17940] (ab203114)

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.

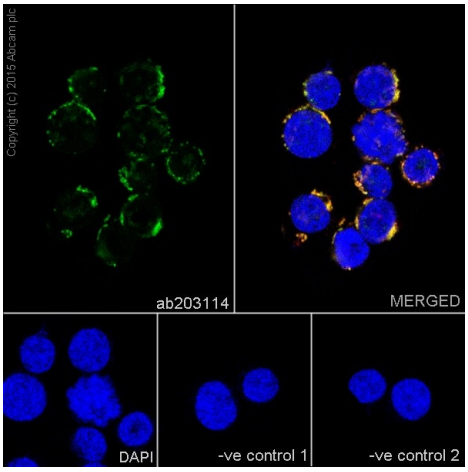


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HADHA antibody [EPR17940] (ab203114)

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.

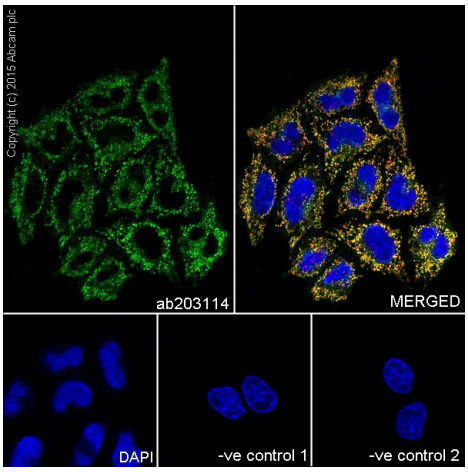


Immunocytochemistry/ Immunofluorescence - Anti-HADHA antibody [EPR17940] (ab203114)

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.

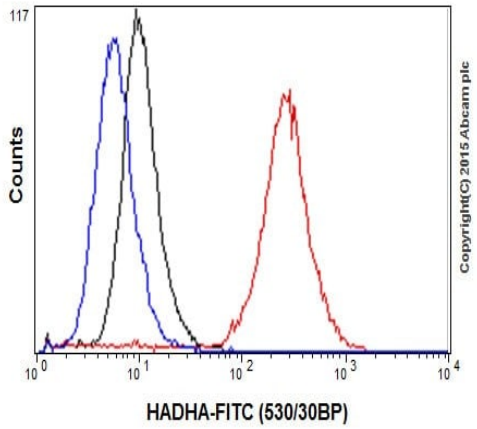


Immunocytochemistry/ Immunofluorescence - Anti-HADHA antibody [EPR17940] (ab203114)

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.

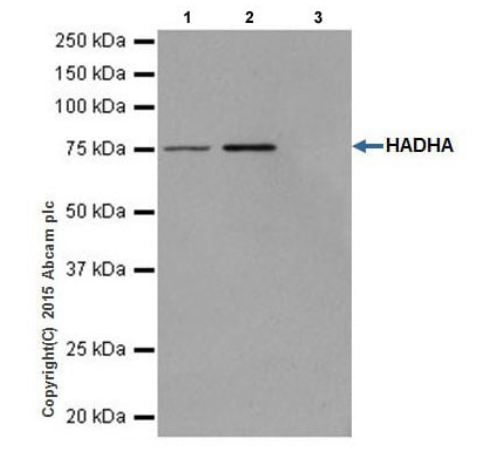
The nuclear counter stain is DAPI (blue). COX IV is detected with **ab33985** (anti-COX IV Mitochondrial Marker mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

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)

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.



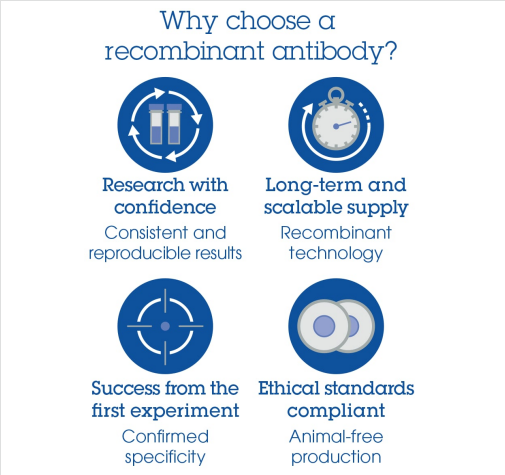
Immunoprecipitation - Anti-HADHA antibody [EPR17940] (ab203114)

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.



Anti-HADHA antibody [EPR17940] (ab203114)

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