abcam

ab227997

Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free

Recombinant | RabMAb | Advanced Validation | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR3648
Purification technique	Affinity purification Protein A
Concentration	1.002 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	-

Notes	-

ChIP-seq

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

IP

Not recommended

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.

Target data

See full target information HNF4A

Function Transcriptional regulator which controls the expression of hepatic genes during the transition of endodermal cells to hepatic progenitor cells, facilitating the recruitment of RNA pol II to the promoters of target genes (PubMed:30597922). Activates the transcription of CYP2C38 (By similarity). Represses the CLOCK-BMAL1 transcriptional activity and is essential for circadian rhythm maintenance and period regulation in the liver and colon cells (PubMed:30530698).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab227997 is the carrier-free version of ab92378.

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species.

Please contact us for more information.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

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.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

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

Activity summary	HNF-4-alpha also known as hepatocyte nuclear factor 4-alpha (HNF4A) is a nuclear protein that functions as a transcription factor. It plays an important role in regulating gene expression in the liver kidney and intestine. HNF-4-alpha has a molecular mass of about 54 kDa and is expressed in tissues where it maintains cellular differentiation and metabolic homeostasis. In cellular assays such as ELISA and CHIP detection of HNF-4-alpha provides insights into its expression levels and activity in various biological contexts.
Biological function summary	HNF-4-alpha influences the expression of genes involved in liver and pancreatic function. It acts as a transcriptional regulator that controls fatty acid oxidation gluconeogenesis and insulin secretion. HNF-4-alpha interacts with other proteins to form transcriptional complexes that fine-tune the expression of metabolic enzymes and transport proteins. This protein is key to ensuring that metabolic genes are expressed in a coordinated manner which is essential for maintaining normal metabolic processes.
Pathways	HNF-4-alpha participates in the insulin signaling and lipid metabolism pathways. Through these pathways it interacts with other transcription factors such as HNF-1-alpha and PPAR-alpha which coordinate the regulation of genes involved in glucose metabolism and fatty acid oxidation. HNF-4-alpha plays a critical role by modulating the expression of enzymes like gluconeogenic enzymes in response to metabolic needs which connects it to key metabolic pathways influencing overall energy homeostasis.
Associated diseases and disorders	HNF-4-alpha is linked to maturity-onset diabetes of the young (MODY1) and certain types of liver diseases. Mutations or dysregulation in HNF-4-alpha can affect the expression of genes essential for insulin production and glucose metabolism leading to disorders like diabetes. Additionally HNF-4-alpha interacts with other proteins and transcription factors implicated in these conditions such as HNF-1-alpha. Understanding the role of HNF-4-alpha in these diseases can provide insights into potential therapeutic targets and intervention strategies.

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.

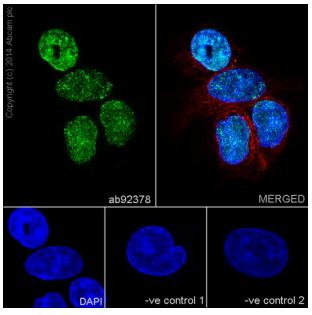
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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:
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9 product images



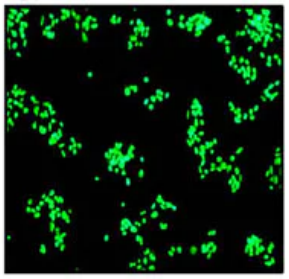
Immunocytochemistry/ Immunofluorescence - Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free (ab227997)

HNF-4-alpha Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HNF-4-alpha antibody

Immunocytochemistry/Immunofluorescence analysis of HepG2 cells labelling HNF-4 with purified ab92378 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/500) and ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500) were also used.
Control 1: primary antibody (1/100) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: ab7291 (1/1000) and secondary antibody, ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).

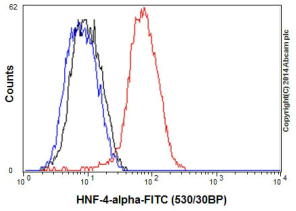
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92378).



Immunocytochemistry/ Immunofluorescence - Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free (ab227997)

HNF-4-alpha Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HNF-4-alpha antibody

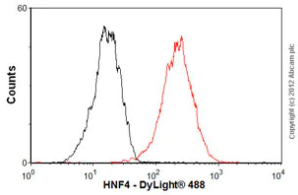
Immunocytochemistry/Immunfluorescence analysis of HepG2 cells labelling HNF-4-alpha with unpurified ab92378 at a 1/100 dilution.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92378).



Flow Cytometry (Intracellular) - Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free (ab227997)

HNF-4-alpha Flow Cytometry (Intracellular) staining using rabbit Anti-HNF-4-alpha antibody

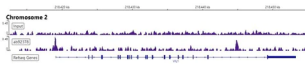
Intracellular Flow Cytometry analysis of HepG2 cells labelling HNF-4 with purified ab92378 at 1/70 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92378).



Flow Cytometry (Intracellular) - Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free (ab227997)

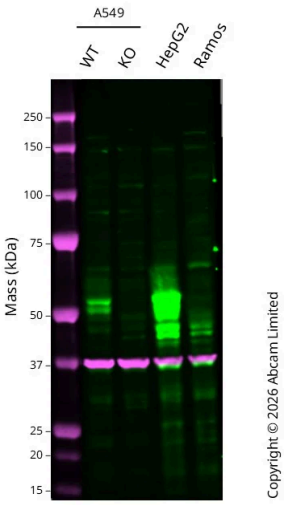
HNF-4-alpha Flow Cytometry (Intracellular) staining using rabbit Anti-HNF-4-alpha antibody

Overlay histogram showing HepG2 cells stained with unpurified ab92378 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (unpurified ab92378, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/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 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92378).



ChIP-sequencing - Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free (ab227997)

Chromatin was prepared from HepG2 (Human liver hepatocellular carcinoma cell line) cells. ChIP was performed with 10^7 HepG2 cells and 8 µg of ab92378 [EPR3648]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92378).



Western blot - Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free (ab227997)

HNF-4-alpha Western blot staining using rabbit Anti-HNF-4-alpha antibody

This data was developed using ab92378, the same antibody clone in a different buffer formulation.

Western blot: Anti-HNF-4-alpha antibody [EPR3648] ab92378 staining at 1/2000 dilution, shown in green; Mouse anti GAPDH ab8245 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 53 kDa in Wild-type A549 cell lysates with no signal observed at this size in HNF4A knockout A549 cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5% Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-HNF-4-alpha antibody [EPR3648] (ab92378) at 1/2000 dilution

Lane 1:
Wild-type A549 at 20 µg

Lane 2:
Western blot - Human HNF4A knockout A549 cell line (ab288931) at 20 µg

Lane 3:
HepG2 at 20 µg

Lane 4:
HEK-293 at 20 µg

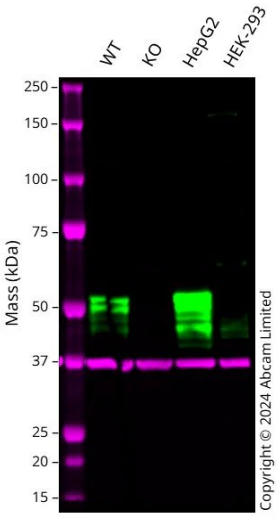
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 53 kDa

Observed band size: 53 kDa



Western blot - Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free (ab227997)

HNF-4-alpha Western blot staining using rabbit Anti-HNF-4-alpha antibody

This data was developed using the same antibody clone in a different buffer formulation (ab92378).

Western blot: Anti-HNF4A antibody [EPR3648] (ab92378) staining at 1/2000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab92378 was shown to bind specifically to HNF4A. A band was observed at 50-55 kDa in wild-type A549 cell lysates with no signal observed at this size in HNF4A knockout cell line. To generate this image, wild-type and HNF4A knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then

imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-HNF-4-alpha antibody [EPR3648] (ab92378) at 1/2000 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
HNF4A knockout A549 cell lysate at 20 µg

Lane 3:
HepG2 cell lysate at 20 µg

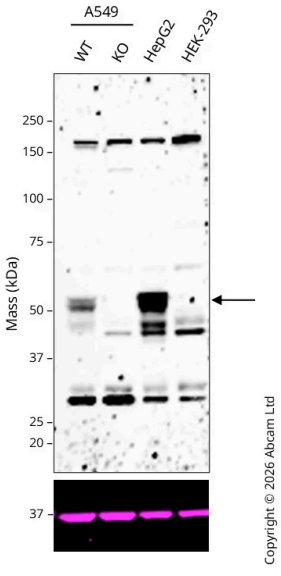
Lane 4:
HEK-293 cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 50 kDa, 55 kDa



Western blot - Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free (ab227997)

HNF-4-alpha Western blot staining using rabbit Anti-HNF-4-alpha antibody

This data was developed using the same antibody clone in a different buffer formulation (ab305721).

Western blot: HRP Anti-HNF-4-alpha antibody [EPR3648] ab305721 staining at 1/1000 dilution, shown in black; Mouse anti GAPDH ab8245 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 53 kDa in Wild-type A549 cell line with no signal observed at this size in HNF4A knockout A549 cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5% Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibody used was Goat anti-Mouse 680RD at 1/20,000 dilution.

All lanes:
Western blot - HRP Anti-HNF-4-alpha antibody [EPR3648] (ab305721) at 1/1000 dilution

Lane 1:
Wild-type A549 at 20 µg

Lane 2:
Western blot - Human HNF4A knockout A549 cell line (ab288931) at 20 µg

Lane 3:
HepG2 at 20 µg

Lane 4:
HEK-293 at 20 µg

Secondary

All lanes:
Goat anti-Mouse 680RD at 1/20000 dilution

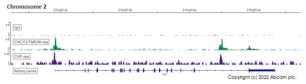
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 53 kDa

Observed band size: 53 kDa

Exposure time: 15s



ChIC/CUT&RUN sequencing - Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free (ab227997)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5 x 10^5 HepG2 (Human liver hepatocellular carcinoma cell line) cells and 5 µg of ab92378 [EPR3648]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control ab172730 is also shown. The ChIP data was conducted on chromatin prepared from HepG2 cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 HepG2 cells and 8 µg of ab92378. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads. Additional screenshots of mapped reads can be downloaded here. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92378).

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