abcam

ab92378

Anti-HNF-4-alpha antibody [EPR3648]

Anti-HNF-4-alpha antibody [EPR3648] (ab92378) is a rabbit monoclonal antibody detecting HNF-4-alpha in **Western Blot, Flow Cytometry (Intra), IP, ICC/IF, ChIP-seq, ChIC/CUT&RUN-seq**. Suitable for **Human**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 10 publications
- Trusted since 2010

Recombinant | RabMAb | Advanced Validation | 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	EPR3648
Purification technique	Affinity purification Protein A
Concentration	0.514 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	5 µg
Notes	-

ChIP-seq

Tested

Species	Human
Dilution info	-
Notes	-

IP

Not recommended

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/2000
Notes	**For unpurified use at 1/1000 - 1/10000.**

ICC/IF

Tested

Species	Human
Dilution info	1/100 - 1/250
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/70
Notes	For unpurified use at 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

IHC-P

Not recommended

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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	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

What is this antibody validated in?
Anti-HNF-4-alpha antibody [EPR3648] (ab92378) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunocytochemistry/immunofluorescence (ICC/IF), ChIP in Human samples.

What is the molecular weight of HNF-4-alpha?
Anti-HNF-4-alpha [EPR3648] (ab92378) specifically detects a band for HNF-4-alpha (UniProt: P41235) at a molecular weight of 53kDa.

Trusted by the scientific community
Anti-HNF-4-alpha [EPR3648] (ab92378) was first used in a scientific publication in 2010 and has been cited over 10 times in peer-reviewed journals.

Other related products
We have a range of other formats of antibody clone [EPR3648] also available for your convenience: ab92378, Alexa Fluor® 488 - ab216897, Alexa Fluor® 647 - ab217073, Carrier free - ab227997, PE - ab305719, APC - ab305720, HRP - ab305721, Alkaline Phosphatase - ab308718, Alexa Fluor® 594 - ab310436, Alexa Fluor® 555 - ab311963, Alexa Fluor® 568 - ab312435, Alexa Fluor® 750 - ab321753

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.

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.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

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

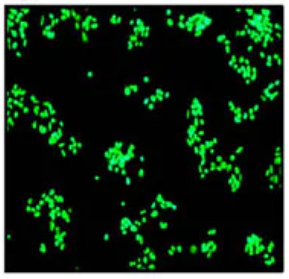
We do not recommend this combination. It is not covered by our product promise.

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:
Terms & Conditions.

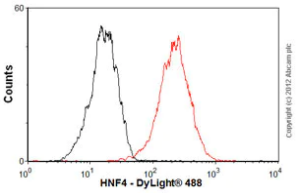
12 product images



Immunocytochemistry/ Immunofluorescence - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

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

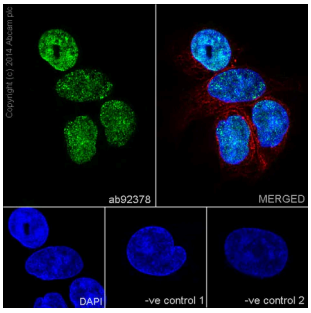
Immunocytochemistry/Immunfluorescence analysis of HepG2 cells labelling HNF-4-alpha with unpurified ab92378 at a 1/100 dilution.



Flow Cytometry (Intracellular) - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

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

Overlay histogram showing HepG2 cells stained with unpurifiedab92378 (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.

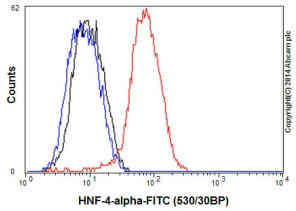


Immunocytochemistry/ Immunofluorescence - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

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).

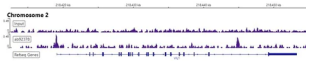
Control 2: ab7291 (1/1000) and secondary antibody, ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).



Flow Cytometry (Intracellular) - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

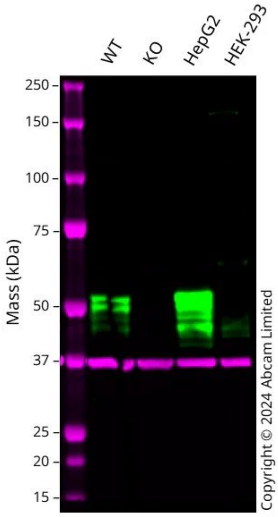
HNF-4-alpha Flow Cytometry (Intracellular) staining using rabbit Anti-HNF-4-alpha antibody

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.



ChIP-sequencing - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

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.



Western blot - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

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

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

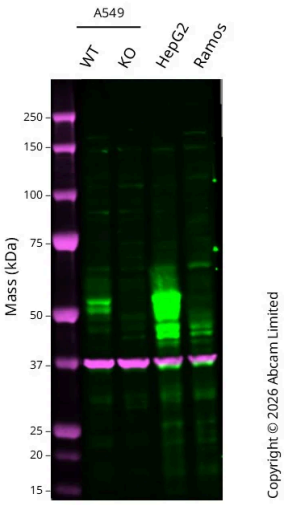
Lane 4:
HEK-293 cell lysate at 20 µg

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



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Western blot - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

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

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

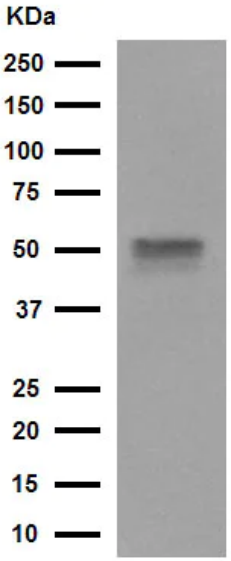
Secondary

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



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Western blot - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

Blocking and dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-HNF-4-alpha antibody [EPR3648] (ab92378) at 1/5000 dilution

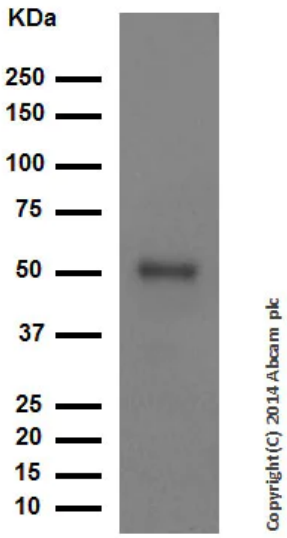
All lanes:
HepG2 cell lysate at 10 µg

Secondary

All lanes:
Peroxidase conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 53 kDa

Observed band size: 53 kDa



Western blot - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

Blocking and dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-HNF-4-alpha antibody [EPR3648] (ab92378) at 1/2000 dilution

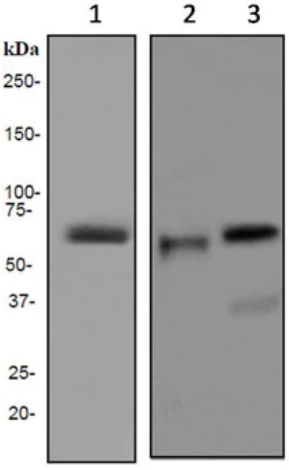
All lanes:
SW480 cell lysate at 20 µg

Secondary

All lanes:
Peroxidase conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 53 kDa

Observed band size: 53 kDa



Western blot - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

All lanes:
Western blot - Anti-HNF-4-alpha antibody [EPR3648] (ab92378) at 1/1000 dilution

Lane 1:
HepG2 cell lysate at 10 µg

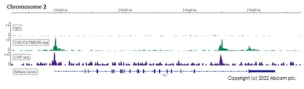
Lane 2:
A549 cell lysate at 10 µg

Lane 3:
SW480 cell lysate at 10 µg

Secondary

All lanes:
HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

Predicted band size: 53 kDa



ChIC/CUT&RUN sequencing - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

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.

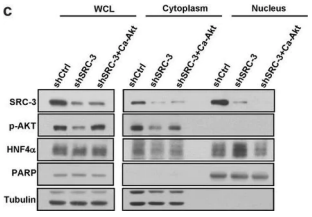


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Western blot - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

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

HNF-4-alpha western blot using anti-HNF-4-alpha antibody [EPR3648] ab92378. Publication image and figure legend from Li, M., Wang, Y., et al., 2019, Cell Biosci, PubMed 31417670.

ab92378 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 ab92378 please see the product overview.

SRC-3 inhibits HNF4α-induced HBV core-promoter activity. a Ectopic expression of SRC-3 or CA-Akt inhibited HNF4α-induced HBV core-promoter activity in HepG2 cells. b Transfection of CA-Akt into SRC-3-knockdown cells inhibited HBV core promoter activity. c Transfection of CA-Akt into SRC-3-knockdown cells prevented the nuclear translocation of HNF4α. Control constructs were transfected into control cell or SRC-3-knockdown cells and CA-Akt were transfected into SRC-3-knockdown cells for 48 h, then cell fractionation was carried out and fractions were analyzed by western blot. All experiments were repeated at least 3 times independently. *p < 0.05, **p < 0.01

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