abcam

Product datasheet

Anti-HNF-4-alpha antibody [EPR3648] ab92378



★★★★★ 4 Abreviews 14 References 12 Images

Overview

Product name Anti-HNF-4-alpha antibody [EPR3648]

Description Rabbit monoclonal [EPR3648] to HNF-4-alpha

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, ICC/IF, Flow Cyt (Intra), ChIC/CUT&RUN-seq, ChIP-sequencing

Unsuitable for: IP

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HepG2, A549 and SW480 cell lysates. IHC-P: Human colon and kidney tissues. ICC/IF:

HepG2 cells. Flow Cyt (intra): HepG2 cells. ChIP-seq: HepG2 cells. ChIC/CUT&RUN-Seq: HepG2

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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

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.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

ClonalityMonoclonalClone numberEPR3648

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab92378 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
WB	★★★★ <u>(1)</u>	1/2000. Predicted molecular weight: 53 kDa. For unpurified use at 1/1000 - 1/10000.
IHC-P	**** (1)	1/250 - 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See IHC antigen retrieval protocols. For unpurified use at 1/100 - 1/250.
ICC/IF	★★★★★ (2)	1/100 - 1/250.
Flow Cyt (Intra)		1/70. For unpurified use at 1/100. <u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ChlC/CUT&RUN-seq		Use at an assay dependent concentration. 5 µg
ChIP-sequencing		Use at an assay dependent concentration.

Application notes Is unsuitable for IP.

Target

Function Transcriptionally controlled transcription factor. Binds to DNA sites required for the transcription of

alpha 1-antitrypsin, apolipoprotein CIII, transthyretin genes and HNF1-alpha. May be essential for

development of the liver, kidney and intestine.

Involvement in diseaseDefects in HNF4A are the cause of maturity-onset diabetes of the young type 1 (MODY1)

[MIM:125850]; also symbolized MODY-1. MODY is a form of diabetes that is characterized by an autosomal dominant mode of inheritance, onset in childhood or early adulthood (usually before 25 years of age), a primary defect in insulin secretion and frequent insulin-independence at the

beginning of the disease.

Sequence similarities Belongs to the nuclear hormone receptor family. NR2 subfamily.

Contains 1 nuclear receptor DNA-binding domain.

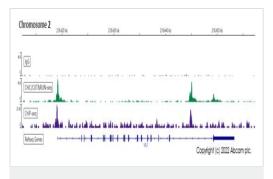
Post-translational Phosphorylated on tyrosine residue(s); phosphorylation is important for its DNA-binding activity.

modifications Phosphorylation may directly or indirectly play a regulatory role in the subnuclear distribution.

Cellular localization

Nucleus.

Images



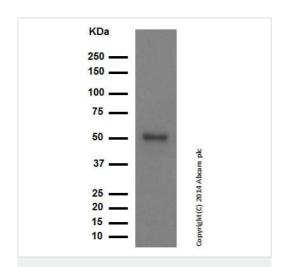
ChIC/CUT&RUN sequencing - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5×10^5 HepG2 (Human liver hepatocellular carcinoma cell line) cells and $5 \mu g$ of ab92378 [EPR3648]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative lgG 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 μ g 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 ChlC (Chromatin Immuno-Cleavage) methods.



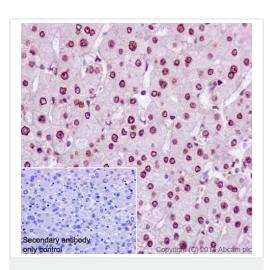
Western blot - Anti-HNF-4-alpha antibody [EPR3648] (ab92378) Anti-HNF-4-alpha antibody [EPR3648] (ab92378) at 1/2000 dilution (purified) + SW480 cell lysate at $20~\mu g$

Secondary

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

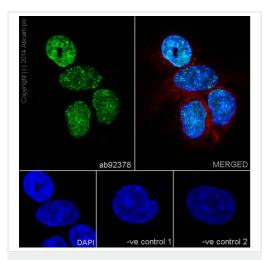
Predicted band size: 53 kDa **Observed band size:** 53 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HNF-4-alpha antibody
[EPR3648] (ab92378)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling HNF-4-alpha with purified ab92378 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

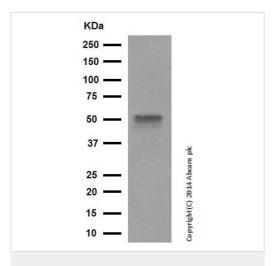


Immunocytochemistry/ Immunofluorescence - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

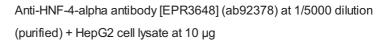
Immunocytochemistry/Immunofluorescence analysis of HepG2 cells labelling HNF-4 with purified ab92378 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor[®] 488-conjugated goat anti-rabbit lgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/500) and ab150120, an Alexa Fluor[®] 594-conjugated goat antimouse lgG (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: <u>ab7291</u> (1/1000) and secondary antibody, <u>ab150077</u>, an Alexa Fluor[®] 488-conjugated goat anti-rabbit lgG (1/500).



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

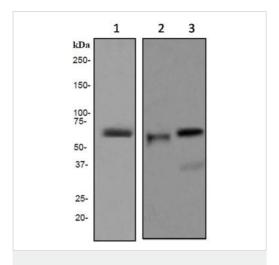


Secondary

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

Predicted band size: 53 kDa **Observed band size:** 53 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



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

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

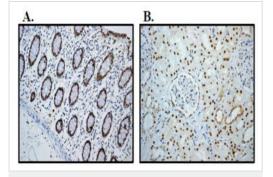
Lane 1 : HepG2 cell lysate
Lane 2 : A549 cell lysate
Lane 3 : SW480 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

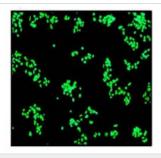
All lanes: HRP-conjugated goat anti-rabbit lgG at 1/2000 dilution

Predicted band size: 53 kDa



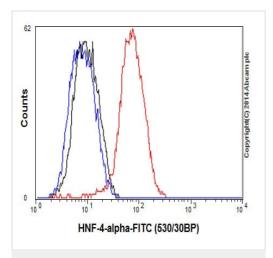
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HNF-4-alpha antibody
[EPR3648] (ab92378)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon tissue (A) and human kidney tissue (B) labelling HNF-4-aplha with unpurified ab92378 at a 1/100 dilution. Detection: DAB staining. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



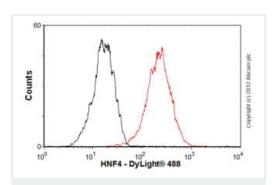
Immunocytochemistry/ Immunofluorescence - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

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



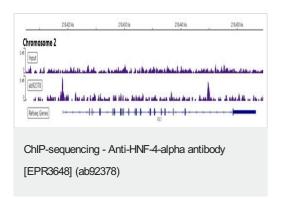
Flow Cytometry (Intracellular) - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

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 lgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal lgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



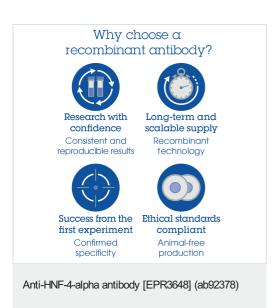
Flow Cytometry (Intracellular) - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

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 lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1µg/1x106 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.



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.

Additional screenshots of mapped reads can be downloaded **here**.



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