abcam

Product datasheet

Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free ab227997

Recombinant RabMAb

9 Images

Overview

Product name	Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free
Description	Rabbit monoclonal [EPR3648] to HNF-4-alpha - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ChIP-sequencing, WB, IHC-P, ICC/IF, ChIC/CUT&RUN-seq **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 cells.
General notes	ab227997 is the carrier-free version of **ab92378**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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. 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. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3648
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab227997 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
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
ChIP-sequencing		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 53 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF		Use at an assay dependent concentration.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.

Application notes Is unsuitable for IP.

Target

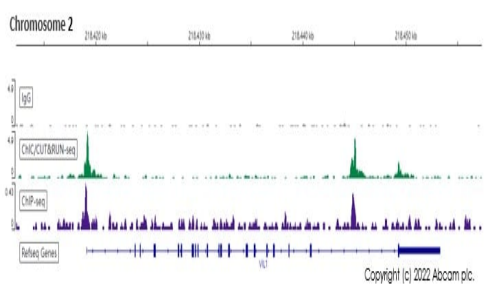
Function Transcriptionally controlled transcription factor. Binds to DNA sites required for the transcription of alpha 1-antitrypsin, apolipoprotein CIII, transthyretin genes and HNF1-alpha. May be essential for development of the liver, kidney and intestine.

Involvement in disease Defects 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 modifications	Phosphorylated on tyrosine residue(s); phosphorylation is important for its DNA-binding activity. Phosphorylation may directly or indirectly play a regulatory role in the subnuclear distribution.
Cellular localization	Nucleus.

Images



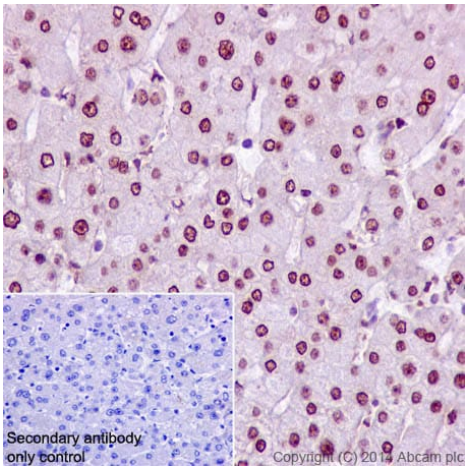
ChIC/CUT&RUN sequencing - Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free (ab227997)

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.

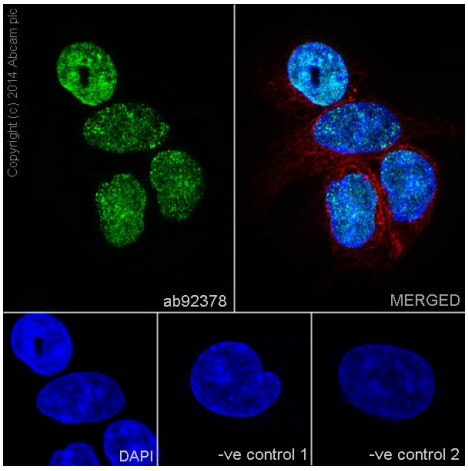
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92378**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free (ab227997)

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.

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



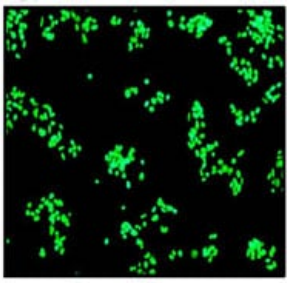
Immunocytochemistry/ Immunofluorescence - Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free (ab227997)

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).

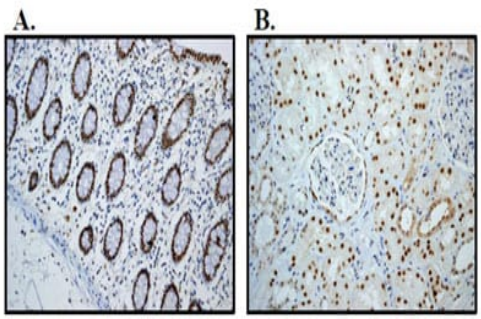
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92378**).



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

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**).

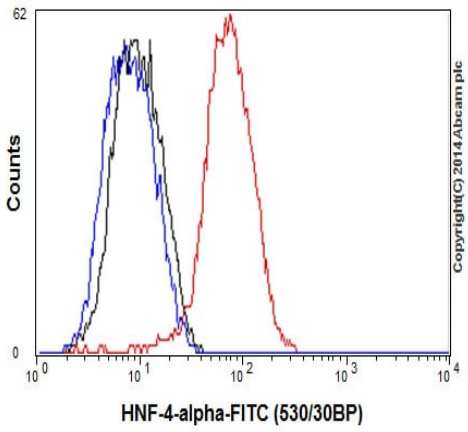


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free (ab227997)

This IHC data was generated using the same anti-HNF4 alpha antibody clone, EPR3648, in a different buffer formulation (cat# **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.

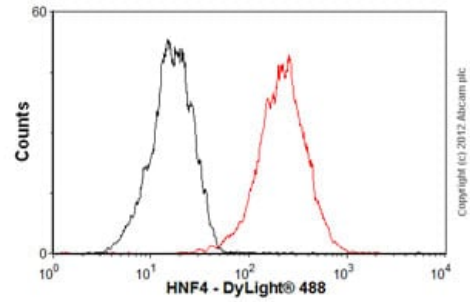
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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

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.

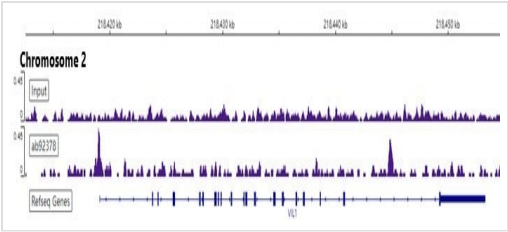
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92378**).



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

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**).

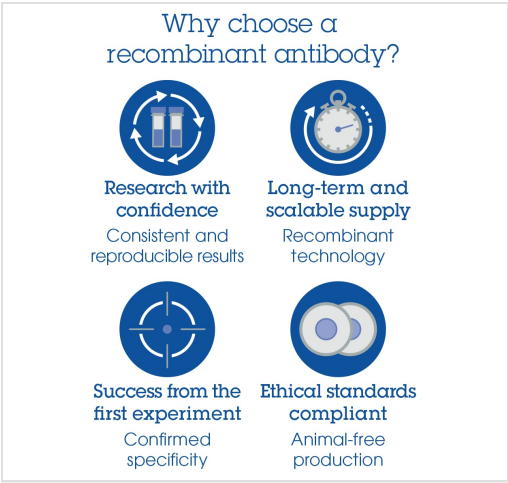


ChIP-sequencing - Anti-HNF-4-alpha antibody [EPR3648] - BSA and Azide free (ab227997)

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.

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

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



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

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