abcam

Product datasheet

Anti-HNF-4-alpha antibody [EPR19265-130] - ChIP Grade ab200142

Recombinant RabMAb

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Overview

Product name	Anti-HNF-4-alpha antibody [EPR19265-130] - ChIP Grade
Description	Rabbit monoclonal [EPR19265-130] to HNF-4-alpha - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, ChIP, IP, Flow Cyt (Intra), ChIC/CUT&RUN-seq
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human colon and fetal liver tissue lysates; HepG2, Caco-2 and SW480 whole cell lysates. ICC/IF: HepG2 and SW480 cells. Flow Cyt (intra): HepG2 cells. ChIP: HepG2 cells. IP: HepG2 whole cell lysate. ChIC/CUT&RUN-Seq: HepG2 cells.
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: 0.05% BSA, 40% Glycerol (glycerin, glycerine), PBS
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR19265-130
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab200142 in the following tested applications.

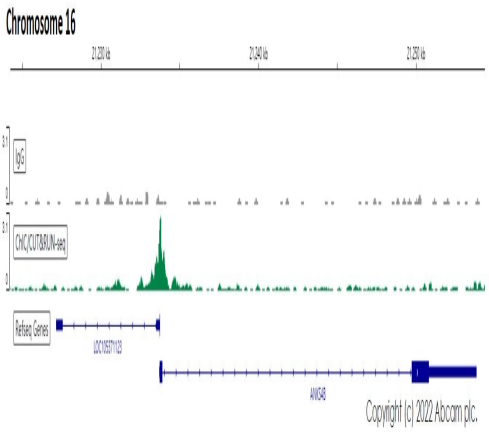
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 53 kDa (predicted molecular weight: 53 kDa).
ICC/IF		1/100.
ChIP		Use 5 µg for 25 µg of chromatin.
IP		1/30.
Flow Cyt (Intra)		1/600.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5 µg

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

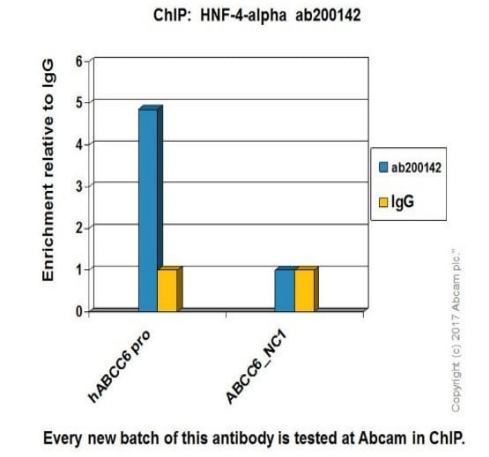


ChIC/CUT&RUN sequencing - Anti-HNF-4-alpha antibody [EPR19265-130] - ChIP Grade (ab200142)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2 x 10^5 HepG2 (Human liver hepatocellular carcinoma cell line) cells and 5 µg of ab200142 [EPR19265-130]. 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.

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

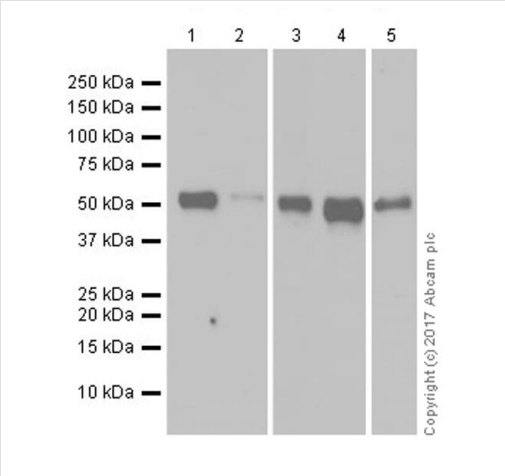
The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



ChIP - Anti-HNF-4-alpha antibody [EPR19265-130] - ChIP Grade (ab200142)

Chromatin was prepared from HepG2 (human hepatocellular carcinoma epithelial cell) cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25 µg of chromatin, 5 µg of ab200142 (blue), and 20 µl of Protein A/G sepharose beads. 5 µg of rabbit normal IgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (SYBR approach).

ChIP was performed according to the literature (PMID: 18850323).



Western blot - Anti-HNF-4-alpha antibody [EPR19265-130] - ChIP Grade (ab200142)

All lanes : Anti-HNF-4-alpha antibody [EPR19265-130] - ChIP Grade (ab200142) at 1/2000 dilution

Lane 1 : Human colon lysate
Lane 2 : Human fetal liver lysate
Lane 3 : HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate
Lane 4 : Caco-2 (human colorectal adenocarcinoma cell line) whole cell lysate
Lane 5 : SW480 (human colorectal adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

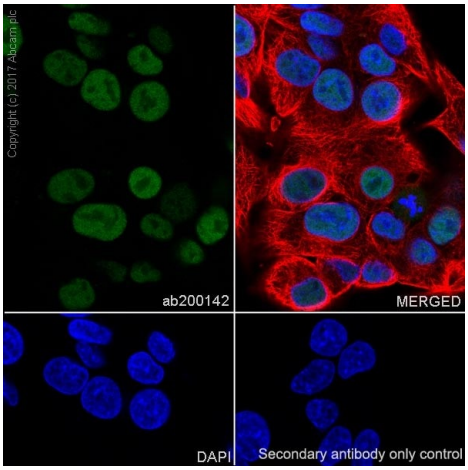
Secondary
All lanes : VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/2000 dilution

Developed using the ECL technique.

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

Exposure times: Lanes 1 and 2: 3 minutes; Lanes 3 and 4: 15 seconds; Lane 5: 3 minutes.

Blocking/Dilution buffer: 5% NFDM/TBST.

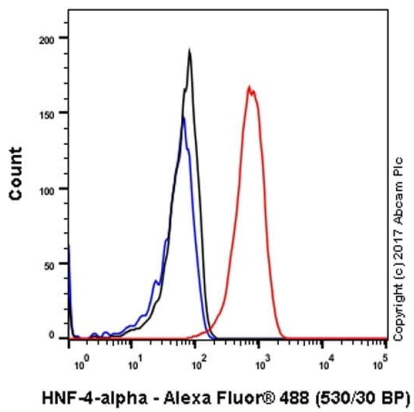


Immunocytochemistry/ Immunofluorescence - Anti-HNF-4-alpha antibody [EPR19265-130] - ChIP Grade (ab200142)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HepG2 (human liver hepatocellular carcinoma cell line) cells labeling HNF-4-alpha with ab200142 at 1/100 dilution, followed by **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 dilution (green). Confocal image showing nuclear staining on HepG2 cell line.

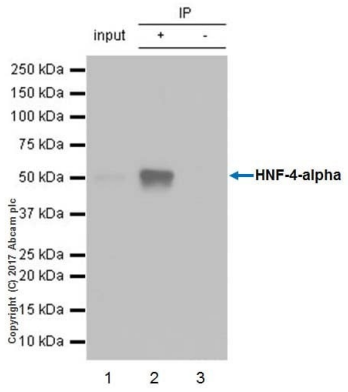
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-HNF-4-alpha antibody [EPR19265-130] - ChIP Grade (ab200142)

Intracellular flow cytometric analysis of4% paraformaldehyde-fixed, 90% methanol-permeabilized HepG2 (human liver hepatocellular carcinoma cell line) cell line labeling HNF-4-alpha with ab200142 at 1/600 (red) compared with Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-HNF-4-alpha antibody [EPR19265-130] - ChIP Grade (ab200142)

HNF-4-alpha was immunoprecipitated from 0.35 mg HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate with ab200142 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab200142 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

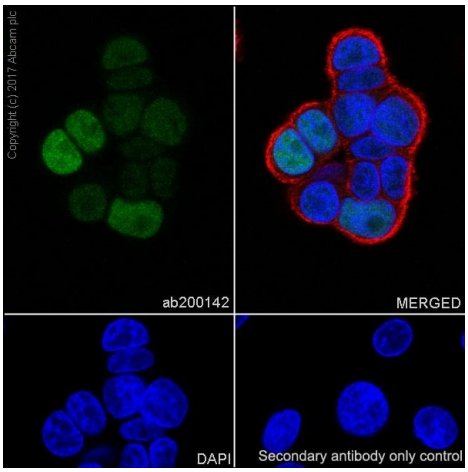
Lane 1: HepG2 (human hepatocellular carcinoma epithelial cell) 10 µg (Input).

Lane 2: ab200142 IP in HepG2 whole cell lysate (+).

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab200142 in HepG2 whole cell lysate (-).

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.

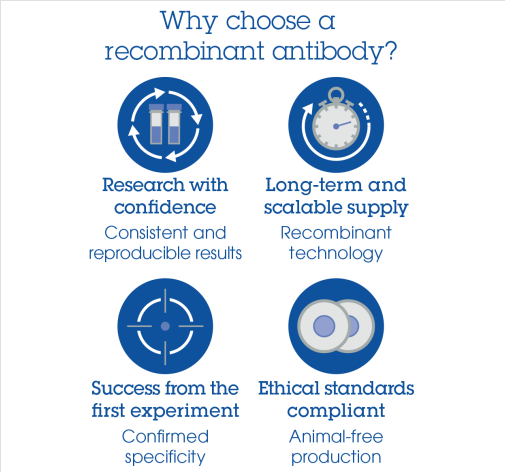


Immunocytochemistry/ Immunofluorescence - Anti-HNF-4-alpha antibody [EPR19265-130] - ChIP Grade (ab200142)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized SW480 (human colorectal adenocarcinoma cell line) cells labeling HNF-4-alpha with ab200142 at 1/100 dilution, followed by **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 dilution (green). Confocal image showing nuclear staining on SW480 cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Anti-HNF-4-alpha antibody [EPR19265-130] - ChIP Grade (ab200142)

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