abcam

Product datasheet

Anti-HNF-4-alpha antibody [EPR16885-99] ab201460

Recombinant RabMAb

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Overview

Product name	Anti-HNF-4-alpha antibody [EPR16885-99]
Description	Rabbit monoclonal [EPR16885-99] to HNF-4-alpha
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt, WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment within Mouse HNF-4-alpha aa 1-200. The exact sequence is proprietary. Database link: P49698
Positive control	WB: HepG2 and Caco-2 whole cell lysates; Human colon and fetal kidney lysates. IHC-P: Human colon, Human liver, mouse liver and rat colon tissues. ICC/IF: HepG2 and HT-29 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	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR16885-99
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab201460** in the following tested applications.

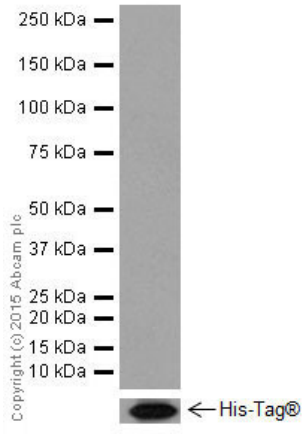
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt		1/1000. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		1/1000. Detects a band of approximately 53 kDa (predicted molecular weight: 53 kDa).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/2000.

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



Western blot - Anti-HNF-4-alpha antibody [EPR16885-99] (ab201460)

Anti-HNF-4-alpha antibody [EPR16885-99] (ab201460) at 1/1000 dilution + Full length mouse HNF-4-gamma recombinant protein at 10 µg

Secondary

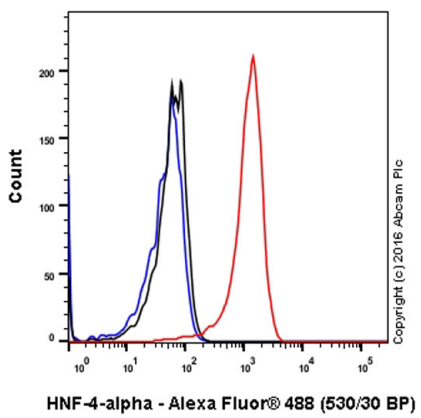
Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 53 kDa

Exposure time: 3 minutes

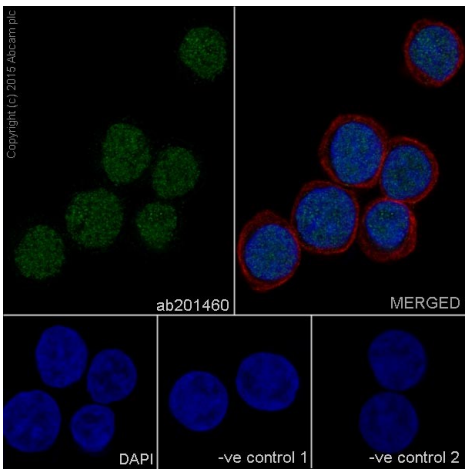
Blocking/Dilution buffer: 5% NFDM/TBST.

Based on sequence analysis of the immunogen, ab201460 shares 72% homology with HNF4 gamma. The levels of XR were tested in the accompanying recombinant protein, WB experiment.



Flow Cytometry - Anti-HNF-4-alpha antibody [EPR16885-99] (ab201460)

Flow Cytometry analysis of HepG2(human hepatocellular carcinoma) labelling CDKN2A/p16INK4a with purified ab201460 at 1/1000 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Alexa Fluor® 488 goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

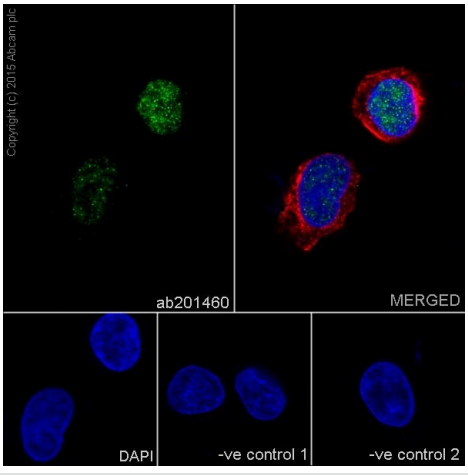


Immunocytochemistry/ Immunofluorescence - Anti-HNF-4-alpha antibody [EPR16885-99] (ab201460)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton-X100 permeabilized HT-29 (Human colorectal adenocarcinoma cells) cells labeling HNF-4-alpha with ab201460 at 1/2000 dilution, followed by AlexaFluor®488 Goat anti-Rabbit secondary antibody (ab150077) at 1/500 dilution (green).

Confocal image showing nuclear staining on HT-29 cell line.

The nuclear counter stain is DAPI (blue). Tubulin is stained with ab7291 anti-Tubulin (mouse mAb) at 1/1000 dilution, followed by AlexaFluor®594 Goat anti-Mouse secondary antibody (ab150120) at 1/500 dilution (red).
-ve control 1: ab201460 at 1/2000 dilution followed by AlexaFluor®594 Goat anti-Mouse secondary antibody (ab150120) at 1/500 dilution.
-ve control 2: ab7291 anti-Tubulin (mouse mAb) at 1/1000 dilution, followed by AlexaFluor®488 Goat anti-Rabbit secondary antibody (ab150077) at 1/500 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-HNF-4-alpha antibody [EPR16885-99] (ab201460)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton-X100 permeabilized HepG2 (Human liver hepatocellular carcinoma) cells labeling HNF-4-alpha with ab201460 at 1/2000 dilution, followed by AlexaFluor®488 Goat anti-Rabbit secondary antibody (ab150077) at 1/500 dilution (green).

Confocal image showing nuclear staining on HT-29 cell line.

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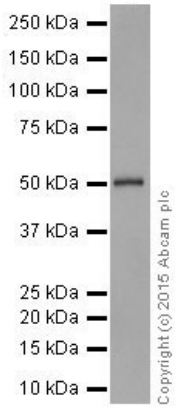


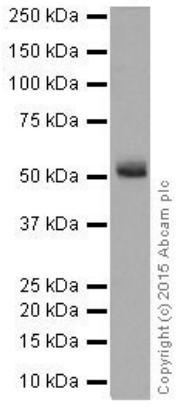
Western blot - Anti-HNF-4-alpha antibody [EPR16885-99] (ab201460)

Anti-HNF-4-alpha antibody [EPR16885-99] (ab201460) at 1/10000 dilution + HepG2 (Human liver hepatocellular carcinoma) whole cell lysate at 10 µg

Secondary
Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

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

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-HNF-4-alpha antibody [EPR16885-99] (ab201460)

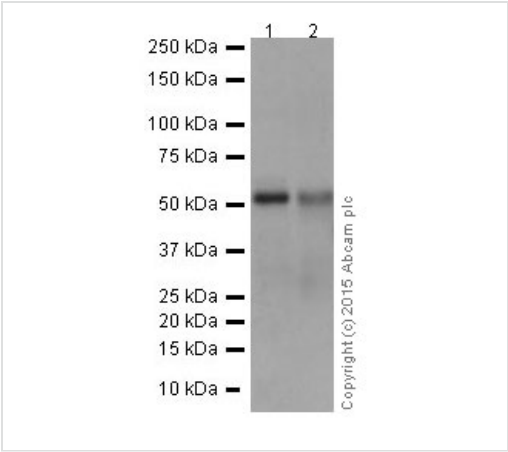
Anti-HNF-4-alpha antibody [EPR16885-99] (ab201460) at 1/1000 dilution + Caco-2 (Human colorectal adenocarcinoma cells) whole cell lysate at 10 µg

Secondary
Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

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

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-HNF-4-alpha antibody [EPR16885-99] (ab201460)

All lanes : Anti-HNF-4-alpha antibody [EPR16885-99] (ab201460) at 1/1000 dilution

Lane 1 : Human colon lysate
Lane 2 : Human fetal kidney lysate

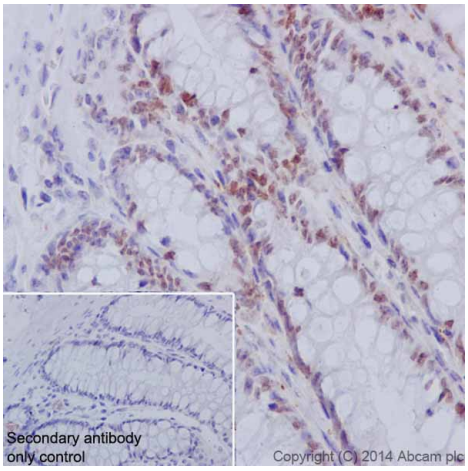
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

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

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.



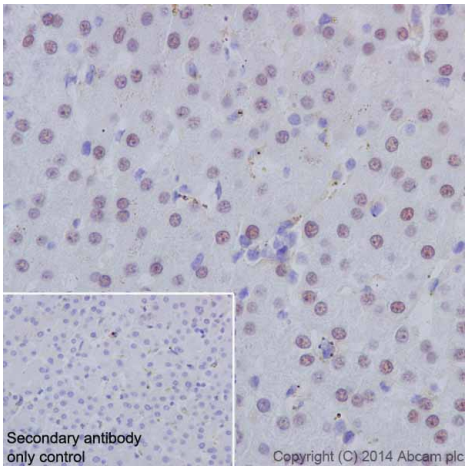
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HNF-4-alpha antibody [EPR16885-99] (ab201460)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling HNF-4-alpha with ab201460 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution.

Nuclear staining on Human colon tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



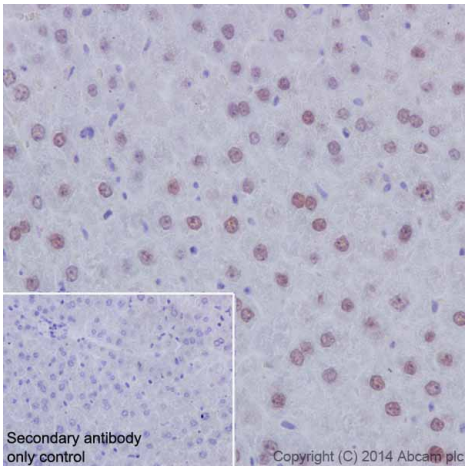
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HNF-4-alpha antibody [EPR16885-99] (ab201460)

Immunohistochemical analysis of paraffin-embedded Human liver tissue labeling HNF-4-alpha with ab201460 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution.

Nuclear staining on Human liver tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



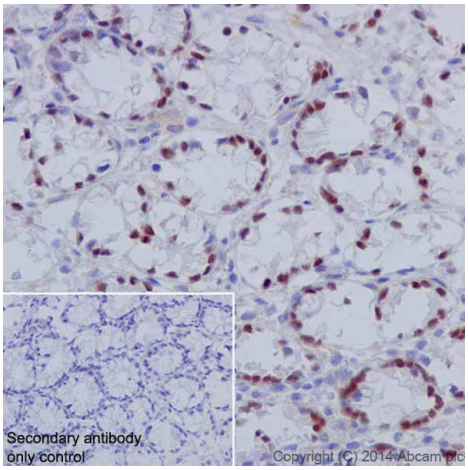
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HNF-4-alpha antibody [EPR16885-99] (ab201460)

Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling HNF-4-alpha with ab201460 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution.

Nuclear staining on mouse liver tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HNF-4-alpha antibody [EPR16885-99] (ab201460)

Immunohistochemical analysis of paraffin-embedded Rat colon tissue labeling HNF-4-alpha with ab201460 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution.

Nuclear staining on rat colon tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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