# abcam

## Product datasheet

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

יעלאעבע RabMAb

★★★★★ 4 Abreviews 14 References 画像数 12

#### 製品の概要

製品名 Anti-HNF-4-alpha antibody [EPR3648]

製品の詳細 Rabbit monoclonal [EPR3648] to HNF-4-alpha

由来種 Rahhit

アプリケーション 適用あり: WB, IHC-P, ICC/IF, Flow Cyt (Intra), ChIC/CUT&RUN-seq, ChIP-sequencing

適用なし: IP

種交差性 交差種: Human

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

ポジティブ・コントロール 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

特記事項 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.

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

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

精製度 Protein A purified

**ポリ**/モノ モノクローナル **ウローン名** EPR3648

アイソタイプ IgG

#### アプリケーション

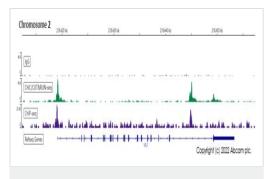
**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab92378の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB	<b>★★★★ (1)</b>	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	<b>★★★★★</b> (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.

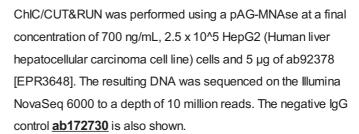
追加情報 Is unsuitable for IP.

#### ターゲット情報

機能 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. 関連疾患 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. 配列類似性 Belongs to the nuclear hormone receptor family. NR2 subfamily. Contains 1 nuclear receptor DNA-binding domain. 翻訳後修飾 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. 細胞内局在 Nucleus.



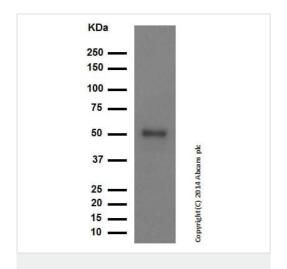
ChIC/CUT&RUN sequencing - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)



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  $\mu$ 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 <u>here</u>.

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



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

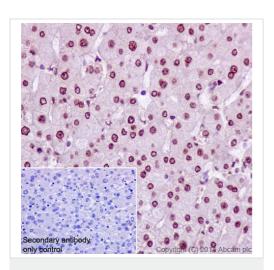
Anti-HNF-4-alpha antibody [EPR3648] (ab92378) at 1/2000 dilution (purified) + SW480 cell lysate at  $20~\mu g$ 

#### **Secondary**

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

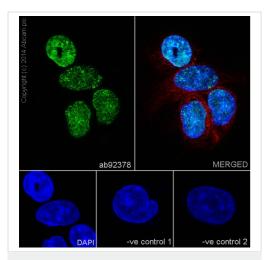
**Predicted band size:** 53 kDa **Observed band size:** 53 kDa

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. <a href="mailto:ab97051">ab97051</a>, 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.

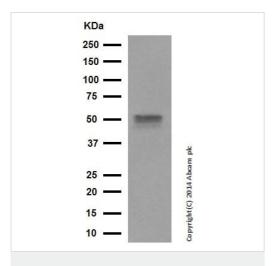


Immunocytochemistry/ Immunofluorescence - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

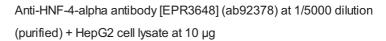
Immunocytochemistry/Immunofluorescence analysis of HepG2 cells labelling HNF-4 with purified ab92378 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. <a href="mailto:ab150077">ab150077</a>, an Alexa Fluor<sup>®</sup> 488-conjugated goat anti-rabbit lgG (1/500)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

Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor<sup>®</sup> 594-conjugated goat anti-mouse IgG (1/500).

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



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

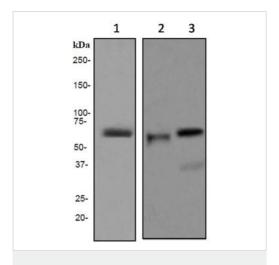


#### Secondary

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)

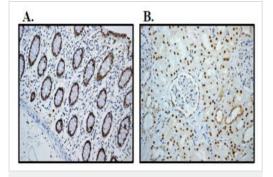
Lane 1 : HepG2 cell lysate
Lane 2 : A549 cell lysate
Lane 3 : SW480 cell lysate

Lysates/proteins at 10 µg per lane.

## Secondary

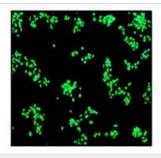
All lanes: HRP-conjugated goat anti-rabbit lgG at 1/2000 dilution

Predicted band size: 53 kDa



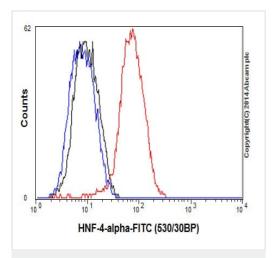
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HNF-4-alpha antibody
[EPR3648] (ab92378)

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.



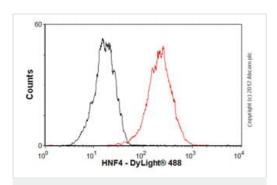
Immunocytochemistry/ Immunofluorescence - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

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



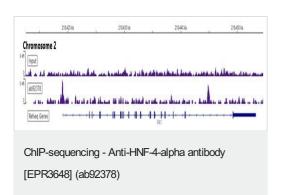
Flow Cytometry (Intracellular) - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

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.



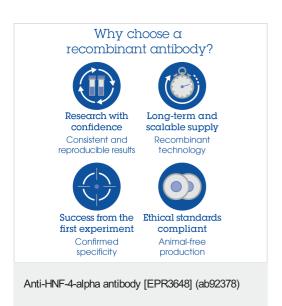
Flow Cytometry (Intracellular) - Anti-HNF-4-alpha antibody [EPR3648] (ab92378)

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  $\mu$ g 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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