# Anti-HNF-4-alpha Antibody [JE63-17] HA721006

| Product Type:  | Recombinant Rabbit monoclonal IgG, primary antibodies   |
|--|---|
| Species reactivity:  | Human, Mouse, Rat   |
| Applications:  | WB, IHC-P, mIHC, IF-Tissue  |
| Molecular Wt:  | Predicted band size: 53 kDa   |
| Clone number:  | JE63-17   |
| Description:   | The protein encoded by this gene is a nuclear transcription factor which binds DNA as a homodimer. The encoded protein controls the expression of several genes, including hepatocyte nuclear factor 1 alpha, a transcription factor which regulates the expression of several hepatic genes. This gene may play a role in development of the liver, kidney, and intestines. Mutations in this gene have been associated with monogenic autosomal dominant non-insulin-dependent diabetes mellitus type I. Alternative splicing of this gene results in multiple transcript variants encoding several different isoforms. |
| lmmunogen:   | Recombinant protein within mouse HNF-4-alpha aa 1-250/474.  |
| Positive control:  | HepG2 cell lysates, mouse liver tissue lysate, rat liver tissue lysate, human liver tissue, mouse liver tissue, rat colon tissue, human colon tissue, mouse colon tissue.   |
| Subcellular location:                                      | Nucleus.  |
| Database links:  | SwissProt: P41235 Human   P49698 Mouse   P22449 Rat   |
| Recommended Dilutions:<br>WB<br>IHC-P<br>mIHC<br>IF-Tissue | 1:500<br>1:400<br>1:2,000-1:5,000<br>1:200  |
| Storage Buffer:  | 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.   |
| Storage Instruction:                                       | Shipped at $4^\circ\!\mathrm{C}$ . Store at +4 $^\circ\!\mathrm{C}$ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20 $^\circ\!\mathrm{C}$ long term.  |
| Purity:  | Protein A affinity purified.  |

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#### Images



**Fig1:** Western blot analysis of HNF-4-alpha on HepG2 cell lysates with Rabbit anti-HNF-4-alpha antibody (HA721006) at 1/500 dilution.

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 30 seconds;

10% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721006) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

**Fig2:** Western blot analysis of HNF-4-alpha on different lysates with Rabbit anti-HNF-4-alpha antibody (HA721006) at 1/500 dilution.

Lane 1: Mouse liver tissue lysate Lane 2: Rat liver tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 53 kDa Observed band size: 45~55 kDa

Exposure time: 2 minutes;

10% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721006) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

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INF-4-alpha

45~55kDa

β-actin

55

40

35· 25·

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Fig3: Fluorescence multiplex immunohistochemical analysis of mouse liver (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-HNF4a (HA721006, Cyan), anti-CK19 (ET1601-6, Magenta) and anti-aSMA (ET1607-53, Yellow) on liver. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immunostaining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of HA721006 (1/5,000 dilution), ET1601-6 (1/10,000 dilution) and ET1607-53 (1/10,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

Fig4: Fluorescence multiplex immunohistochemical analysis of

mouse liver (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Desmin (ET1606-30, White), anti-HNF4α (HA721006, Red) and anti-GS (EM1902-39, Yellow) on liver. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immunostaining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of ET1606-30 (1/800 dilution), HA721006 (1/5,000 dilution) and EM1902-39 (1/2,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Zeiss Observer 7 Inverted Fluorescence Microscope.



**Fig5:** Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-HNF-4-alpha antibody (HA721006) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA721006) at 1/400 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

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Fig6: Fluorescence multiplex immunohistochemical analysis of mouse liver (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Th (ET1611-12, Green), anti-HNF4a (HA721006, Magenta), anti-CK19 (ET1601-6, Cyan), anti- $\alpha$ -sma (ET1607-53, Red) and anti- $\beta$ -catenin (ET1601-5, Yellow) on liver. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immunostaining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of ET1611-12 (1/1,000 dilution), HA721006 (1/2,000 dilution), ET1601-6 (1/3,000 dilution), ET1607-53 (1/10,000 dilution) and ET1601-5 (1/2,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.



**Fig7:** Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-HNF-4-alpha antibody (HA721006) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA721006) at 1/400 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig8:** Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-HNF-4-alpha antibody (HA721006) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA721006) at 1/400 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

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**Fig9:** Immunofluorescence analysis of paraffin-embedded human colon tissue labeling HNF-4-alpha with Rabbit anti-HNF-4-alpha antibody (HA721006) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721006, green) at 1/200 dilution overnight at 4  $^{\circ}$ C, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor M 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).



**Fig10:** Immunofluorescence analysis of paraffin-embedded mouse colon tissue labeling HNF-4-alpha with Rabbit anti-HNF-4-alpha antibody (HA721006) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721006, green) at 1/200 dilution overnight at 4  $^{\circ}$ C, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor M 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).



**Fig11:** Immunofluorescence analysis of paraffin-embedded rat colon tissue labeling HNF-4-alpha with Rabbit anti-HNF-4-alpha antibody (HA721006) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721006, green) at 1/200 dilution overnight at 4  $^{\circ}$ C, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor M 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

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Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

#### **Background References**

- 1. Song H. et. al. HNF4A-AS1/hnRNPU/CTCF axis as a therapeutic target for aerobic glycolysis and neuroblastoma progression. J Hematol Oncol. 2020 Mar
- 2. Zhang X. et. al. circRNA\_104075 stimulates YAP-dependent tumorigenesis through the regulation of HNF4a and may serve as a diagnostic marker in hepatocellular carcinoma. Cell Death Dis. 2018 Oct

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