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, IF-Cell
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.

Immunogen: Recombinant protein within mouse HNF-4-alpha aa 1-250/474.

Positive control: HepG2 cell lysates, 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:

WB	1:1,000
IHC-P	1:400
mIHC	1:100-1:5,000
IF-Tissue	1:200
IF-Cell	1:500

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

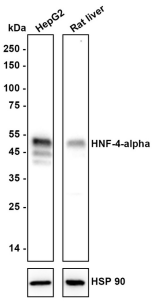

Fig1: Western blot analysis of HNF-4-alpha on different lysates with Rabbit anti-HNF-4-alpha antibody (HA721006) at 1/1,000 dilution.

Lane 1: Hep G2 (Human hepatocellular carcinoma cell) cell lysate (20 µg/Lane)
Lane 2: Rat liver tissue lysate (40 µg/Lane)

Exposure time: 16 seconds; ECL: K1801

Blocking: 5% NFDM/TBST, 1 hour at room temperature
Primary antibody: HA721006, 1/1,000 in 5% NFDM/TBST, overnight at 4 ℃
Secondary antibody: Goat anti-Rabbit IgG-HRP (HA1001), 1/50,000 in 5% NFDM/TBST, 1 hour at room temperature

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

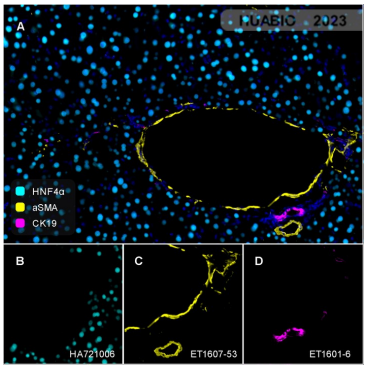

Fig2: Fluorescence multiplex immunohistochemical analysis of mouse liver (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-HNF4α (HA721006, Cyan), anti-CK19 (ET1601-6, Magenta) and anti-aSMA (ET1607-53, Yellow) on liver. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining 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.

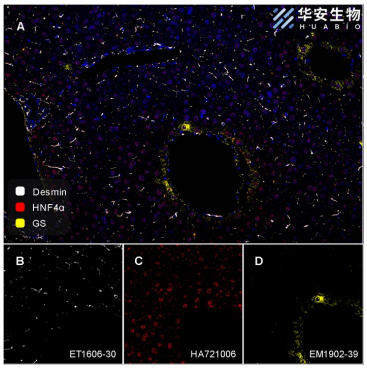

Fig3: Fluorescence multiplex immunohistochemical analysis of mouse liver (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Desmin (ET1606-30, White), anti-HNF4α (HA721006, Red) and anti-GS (EM1902-39, Yellow) on liver. HRP Conjugated UltraPolymer Goat Polyclonal Antibody 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℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Zeiss Observer 7 Inverted Fluorescence Microscope.

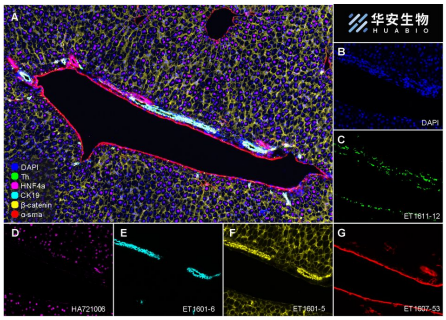

Fig4: Fluorescence multiplex immunohistochemical analysis of mouse liver (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Th (ET1611-12, Green), anti-HNF4a (HA721006, Magenta), anti-CK19 (ET1601-6, Cyan), anti-α-sma (ET1607-53, Red) and anti-β-catenin (ET1601-5, Yellow) 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℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

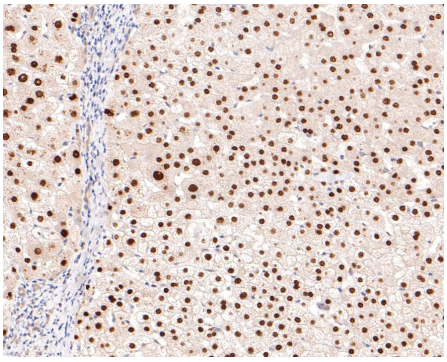

Fig5: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-HNF-4-alpha antibody (HA721006) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O 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.

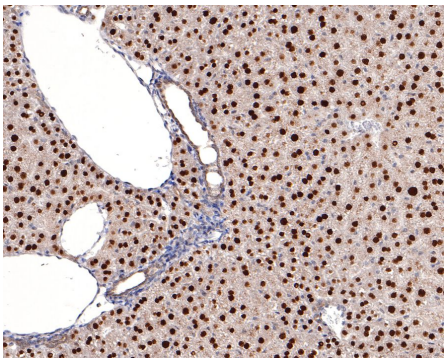

Fig6: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-HNF-4-alpha antibody (HA721006) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O 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.

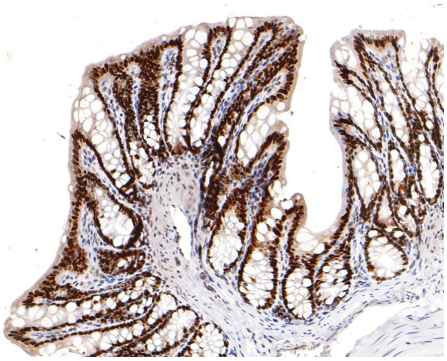

Fig7: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-HNF-4-alpha antibody (HA721006) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O 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.

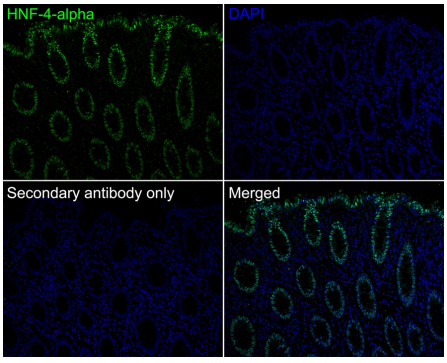

Fig8: Immunofluorescence analysis of paraffin-embedded human colon tissue labeling HNF-4-alpha with Rabbit anti-HNF-4-alpha antibody (HA721006) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) 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 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

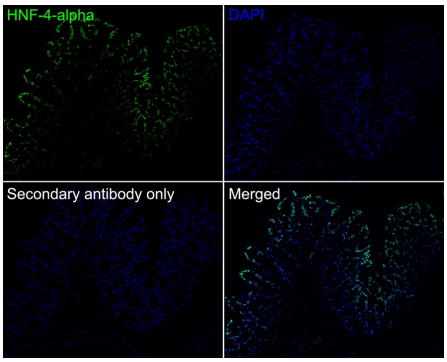

Fig9: Immunofluorescence analysis of paraffin-embedded mouse colon tissue labeling HNF-4-alpha with Rabbit anti-HNF-4-alpha antibody (HA721006) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) 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 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

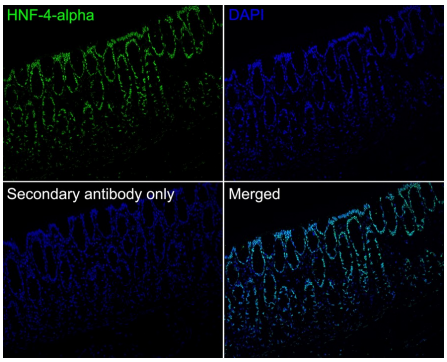

Fig10: Immunofluorescence analysis of paraffin-embedded rat colon tissue labeling HNF-4-alpha with Rabbit anti-HNF-4-alpha antibody (HA721006) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) 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 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

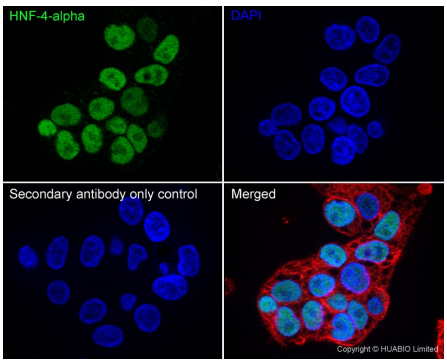

Fig11: Application: Immunocytochemistry (IF-cell)

Species: Human
Sample: Hep G2 (Human hepatocellular carcinoma cell)

Fixation: 4% Paraformaldehyde, 15 minutes at room temperature.
Permeabilization: 0.1% Triton X-100, 15 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 1 hour at room temperature.
Antibody dilution buffer: 1% BSA in PBST.

Primary antibody: HA721006, 1/500, overnight at 4℃.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 45 minutes at room temperature.
Counterstain: Beta tubulin (HA601187, Red), 1/100, overnight at 4℃. The nuclear counterstain was DAPI (Blue).

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

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