Anti-HNF-4-alpha Antibody [SN72-03]

ET1611-43

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 53 kDa
Clone number:	SN72-03

Description: HNF-4 alpha is a transcription factor that binds DNA as a homodimer. HNF-4 alpha is important in liver, kidney, and intestinal development. It has also been intensely studied as one of a variety of genes responsible for diabetes mellitus. HNF-4 alpha has been shown in knock out mice to be essential for the morphogenic and functional differentiation of hepatocytes. HNF-4 alpha is a dominant regulator of epithelial phenotypes able to drive the mesenchymal-to-epithelial transition when expressed in fibroblasts.

Immunogen: Synthetic peptide within Human HNF-4-alpha aa 21-70 / 474.

Positive control: A549 cell lysates, LOVO cell lysates, HepG2, SW480, human colon cancer tissue, human small intestine tissue, mouse colon tissue, rat colon tissue, Mouse liver tissue lysate, Mouse kidney tissue lysate, Mouse brain tissue lysate.

Subcellular location: Nucleus.

Database links: SwissProt: P41235 Human | P49698 Mouse | P22449 Rat

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:100
IHC-P	1:200-1:3,000
FC	1:1,000

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). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

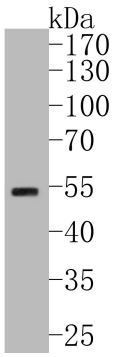

Fig1: Western blot analysis of HNF-4-alpha on A549 cell lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1611-43, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

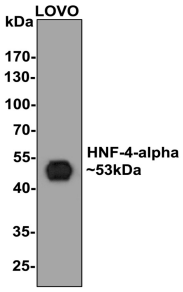

Fig2: Western blot analysis of HNF-4-alpha on LOVO cell lysates with Rabbit anti-HNF-4-alpha antibody (ET1611-43) at 1/500 dilution.

Lysates/proteins at 10 µg/Lane.

Predicted band size: 53 kDa
Observed band size: 53 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 (ET1611-43) 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.

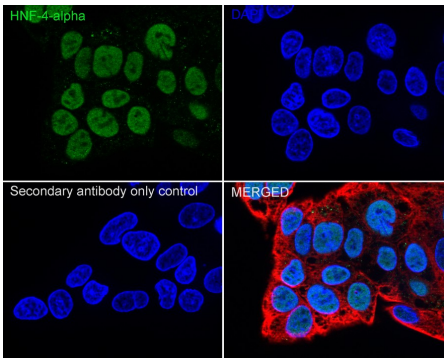

Fig3: Immunocytochemistry analysis of HepG2 cells labeling HNF-4-alpha with Rabbit anti-HNF-4-alpha antibody (ET1611-43) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-HNF-4-alpha antibody (ET1611-43) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

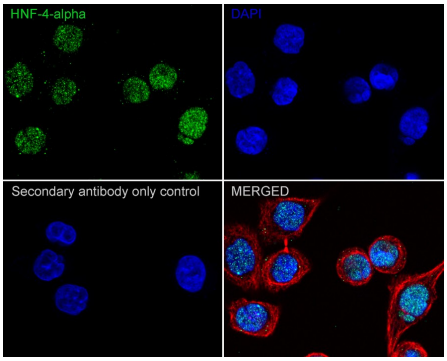

Fig4: Immunocytochemistry analysis of SW480 cells labeling HNF-4-alpha with Rabbit anti-HNF-4-alpha antibody (ET1611-43) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-HNF-4-alpha antibody (ET1611-43) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

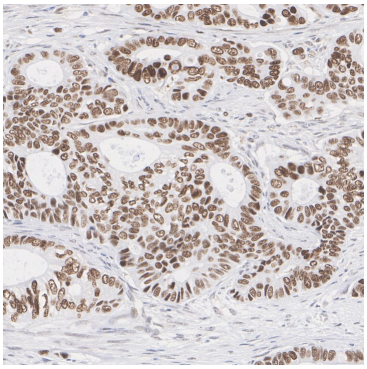

Fig5: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-HNF-4-alpha antibody (ET1611-43) at 1/3,000 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1611-43) at 1/3,000 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.

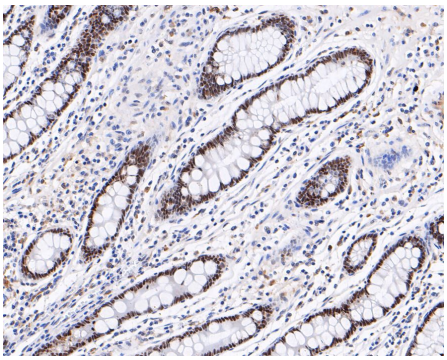

Fig6: Immunohistochemical analysis of paraffin-embedded human small intestine tissue with Rabbit anti-HNF-4-alpha antibody (ET1611-43) at 1/200 dilution.

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 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-43) at 1/200 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.

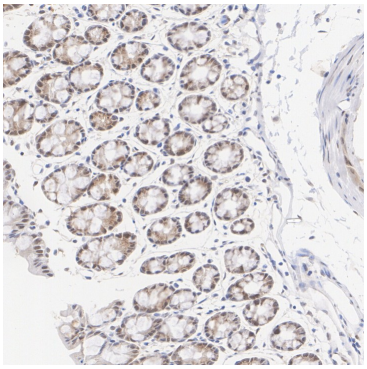

Fig7: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-HNF-4-alpha antibody (ET1611-43) at 1/3,000 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1611-43) at 1/3,000 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.

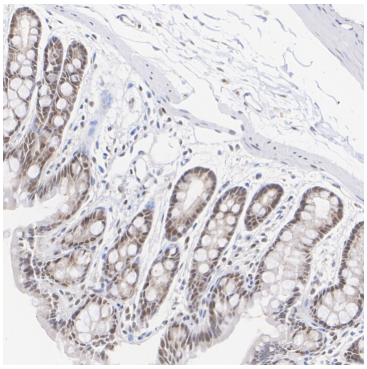

Fig8: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-HNF-4-alpha antibody (ET1611-43) at 1/3,000 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1611-43) at 1/3,000 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.

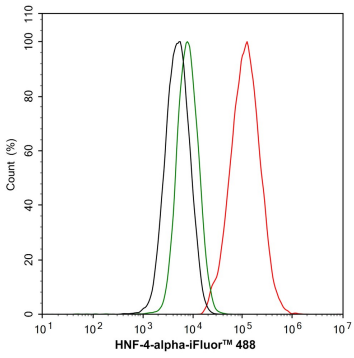

Fig9: Flow cytometric analysis of HepG2 cells labeling HNF-4-alpha.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1611-43, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

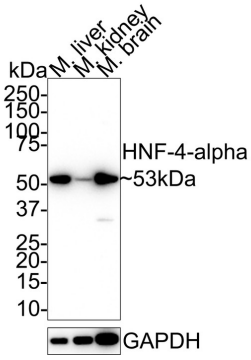

Fig10: Western blot analysis of HNF-4-alpha on different lysates with Rabbit anti-HNF-4-alpha antibody (ET1611-43) at 1/1,000 dilution.

Lane 1: Mouse liver tissue lysate
Lane 2: Mouse kidney tissue lysate
Lane 3: Mouse brain tissue lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 3 minutes;

4-20% 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 (ET1611-43) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Background References

1. Simeone P et al. A unique four-hub protein cluster associates to glioblastoma progression. PLoS One 9:e103030 (2014).
2. Saha SK et al. Mutant IDH inhibits HNF-4a to block hepatocyte differentiation and promote biliary cancer. Nature 513:110-4 (2014).