Anti-NPHS2 Antibody [JB51-33]

ET7107-34

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC, IF-Tissue, mIHC
Molecular Wt:	Predicted band size: 42 kDa
Clone number:	JB51-33

Description: Plays a role in the regulation of glomerular permeability, acting probably as a linker between the plasma membrane and the cytoskeleton. Almost exclusively expressed in the podocytes of fetal and mature kidney glomeruli.

Immunogen: Synthetic peptide within Human NPHS2 aa 334-383 / 383.

Positive control: Rat kidney tissue lysates, human kidney tissue lysate, mouse kidney tissue lysate, human kidney tissue, mouse kidney tissue, rat kidney tissue, 293T.

Subcellular location: Cell membrane. Endoplasmic reticulum.

Database links: SwissProt: Q9NP85 Human | Q91X05 Mouse | Q8K4G9 Rat

Recommended Dilutions:

WB	1:500-1:1,000
IHC-P	1:50-1:200
FC	1:50-1:100
IF-Tissue	1:100
mIHC	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

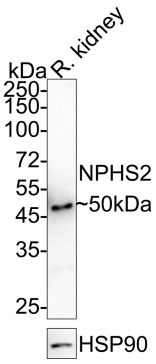

Fig1: Western blot analysis of NPHS2 on rat kidney tissue lysates with Rabbit anti-NPHS2 antibody (ET7107-34) at 1/1,000 dilution.

Lysates/proteins at 20 µg/Lane.

Predicted band size: 42 kDa
Observed band size: 50 kDa

Exposure time: 2 minutes; ECL: K1801;

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 (ET7107-34) 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.

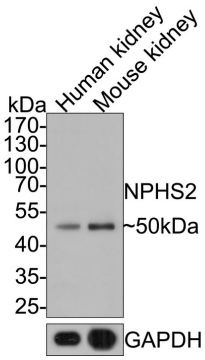

Fig2: Western blot analysis of NPHS2 on different lysates with Rabbit anti-NPHS2 antibody (ET7107-34) at 1/500 dilution.

Lane 1: Human kidney tissue lysate
Lane 2: Mouse kidney tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 42 kDa
Observed band size: 50 kDa

Exposure time: 4 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 (ET7107-34) 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.

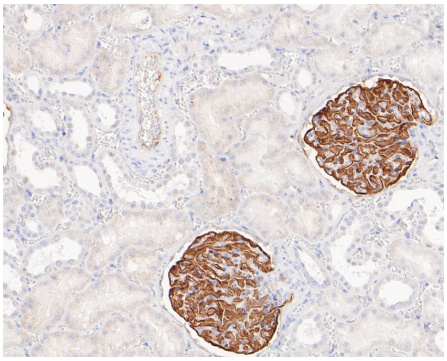

Fig3: Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-NPHS2 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7107-34, 1/200) for 30 minutes 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.

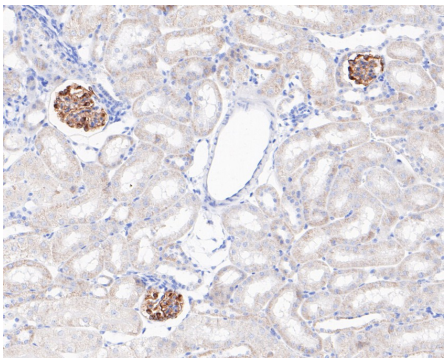

Fig4: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue using anti-NPHS2 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7107-34, 1/200) for 30 minutes 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.

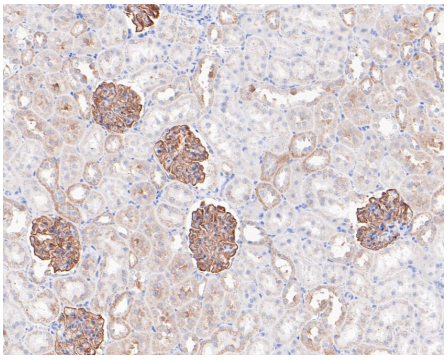

Fig5: Immunohistochemical analysis of paraffin-embedded rat kidney tissue using anti-NPHS2 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7107-34, 1/200) for 30 minutes 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.

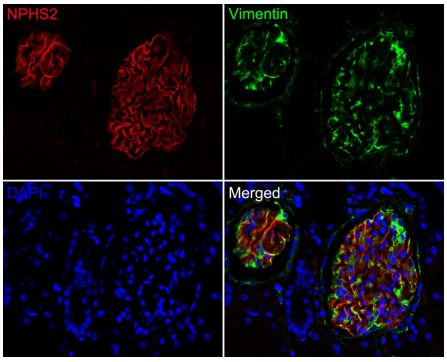

Fig6: Immunofluorescence analysis of paraffin-embedded human kidney tissue labeling NPHS2 (ET7107-34) and Vimentin (EM0401).

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 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 antibodies NPHS2 (ET7107-34, red) at 1/100 dilution and Vimentin (EM0401, green) at 1/400 dilution overnight at 4 ℃, washed with PBS.

iFluor™ 594 conjugate-Goat anti-Rabbit IgG (HA1122) and iFluor™ 488 conjugate-Goat anti-Mouse IgG (HA1125) were used as the secondary antibodies at 1/1,000 dilution. DAPI was used as nuclear counterstain.

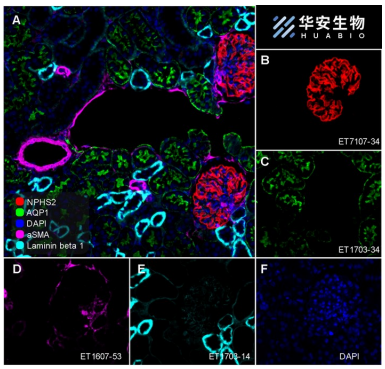

Fig7: Fluorescence multiplex immunohistochemical analysis of mouse kidney (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-NPHS2 (ET7107-34, Red), anti-AQP1 (ET1703-34, Green), anti-Laminin beta 1 (ET1703-14, Cyan) and anti-aSMA (ET1607-53, Magenta) on kidney. HRP Conjugated 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 four rounds of staining: in the order of ET7107-34 (1/1,000 dilution), ET1703-34 (1/5,000 dilution), ET1703-14 (1/1,000 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.

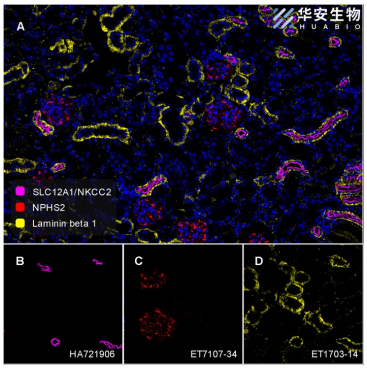

Fig8: Fluorescence multiplex immunohistochemical analysis of mouse kidney (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Laminin beta 1 (ET1703-14, Yellow), anti-NPHS2 (ET7107-34, Red) and anti-SLC12A1/NKCC2 (HA721906, Violet) on kidney. HRP Conjugated UltraPolymer 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 ET1703-14 (1/1,000 dilution), ET7107-34 (1/1,000 dilution) and HA721906 (1/3,000 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.

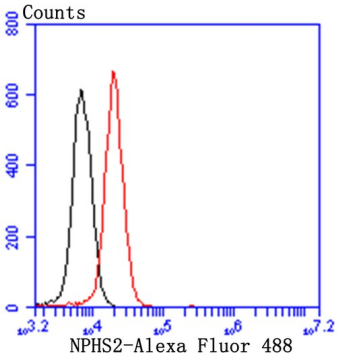

Fig9: Flow cytometric analysis of NPHS2 was done on 293T cells. The cells were fixed, permeabilized and stained with the primary antibody (ET7107-34, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Volker L A et al. Characterization of a short isoform of the kidney protein podocin in human kidney. BMC Nephrol 14:102-102 (2013).
2. Boute N et al. NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. Nat Genet 24:349-354 (2000).