

Anti-NPHS2 Antibody

Catalog Number: PA1322-1

About NPHS2

Podocin(PDCN) is a protein which lines the podocytes and assists in maintaining the barrier at the glomerular basement membrane. NPHS2 is a causative gene for Familial idiopathic nephrotic syndromes, which represents a heterogeneous group of kidney disorders, and include autosomal recessive steroid-resistant nephrotic syndrome, which is characterized by early childhood onset of proteinuria, rapid progression to end-stage renal disease and focal segmental glomerulosclerosis. By positional cloning, NPHS2 was mapped to 1q25-31. It is almost exclusively expressed in the podocytes of fetal and mature kidney glomeruli, and encodes a new integral membrane protein, podocin, belonging to the stomatin protein family. Ten different NPHS2 mutations were found, comprising nonsense, frameshift and missense mutations, to segregate with the disease, demonstrating a crucial role for podocin in the function of the glomerular filtration barrier.

Overview

Product Name	Anti-NPHS2 Antibody
Reactive Species	Human, Mouse, Rat
Description	Rabbit IgG polyclonal antibody for Podocin(NPHS2) detection. Tested with WB, IHC-P, IHC-F in Human;Mouse;Rat.
Application	IHC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg Thimerosal, 0.05mg NaN ₃ .
Storage Instructions	At -20°C for one year. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for a longer time. Avoid repeated freezing and thawing.
Host	Rabbit
Uniprot ID	Q9NP85

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human
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	NPHS2(368-383aa KPVEPLNPKKKDSPML), identical to the related mouse sequence, and different from the related rat sequence by one amino acid.
Predicted Reactive Species	Chicken
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and IHC(F).
Cross Reactivity	No cross reactivity with other proteins
Isotype	N/A
Form	Lyophilized
Concentration	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Immunohistochemistry(Frozen Section), 0.5-1µg/ml, Rat, Human, Mouse Immunohistochemistry(Paraffin-embedded Section), 0.5-1µg/ml, Rat, Human, Mouse, By Heat Western blot, 0.1-0.5µg/ml, Rat, Human, Mouse For protocols please visit https://www.bosterbio.com/protocol-and-troubleshooting/

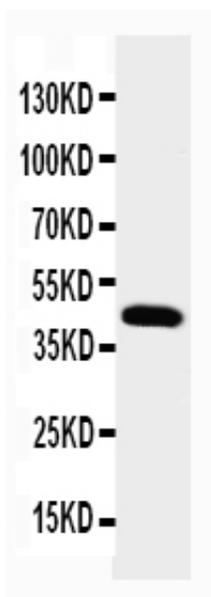
Anti-NPHS2 Antibody (PA1322-1) Images

Figure 1. Western blot analysis of NPHS2 using anti- NPHS2 antibody (PA1322-1).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: rat kidney tissue lysate.

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-NPHS2 antigen affinity purified polyclonal antibody (Catalog # PA1322-1) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for NPHS2 at approximately



45KD. The expected band size for NPHS2 is at 42KD.

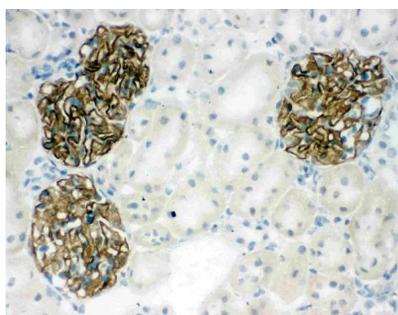


Figure 2. IHC analysis of NPHS2 using anti- NPHS2 antibody (PA1322-1).

NPHS2 was detected in frozen section of rat kidney tissues. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 $\frac{1}{4}$ g/ml rabbit anti-NPHS2 Antibody (PA1322-1) overnight at 4 \AA C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37 \AA C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

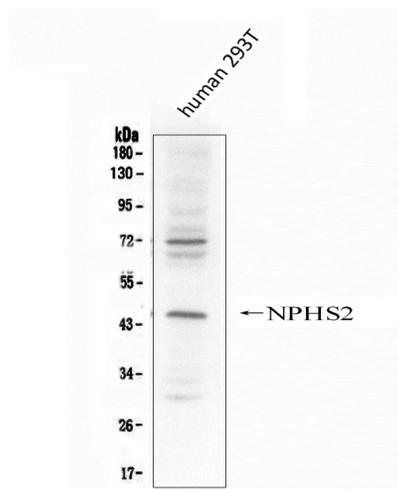


Figure 3. Western blot analysis of NPHS2 using anti- NPHS2 antibody (PA1322-1).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: human 293T whole cell lysates.

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 70 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti- NPHS2 antigen affinity purified polyclonal antibody (Catalog # PA1322-1) at 0.5 $\frac{1}{4}$ g/mL overnight at 4 \AA C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for NPHS2 at approximately 45KD. The expected band size for NPHS2 is at 42KD.

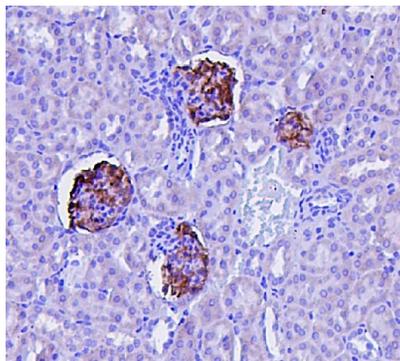


Figure 4. IHC analysis of NPHS2 using anti-NPHS2 antibody (PA1322-1).

NPHS2 was detected in paraffin-embedded section of mouse kidney tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml rabbit anti-NPHS2 Antibody (PA1322-1) overnight at 4 $^{\circ}$ C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37 $^{\circ}$ C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

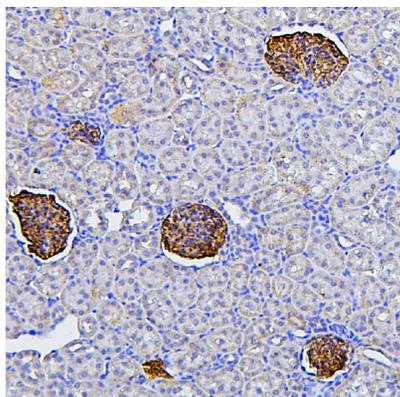


Figure 5. IHC analysis of NPHS2 using anti-NPHS2 antibody (PA1322-1).

NPHS2 was detected in paraffin-embedded section of rat kidney tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml rabbit anti-NPHS2 Antibody (PA1322-1) overnight at 4 $^{\circ}$ C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37 $^{\circ}$ C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

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