ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Nephrin Subunit Antibodies

Cat. # NPHN11-P	Rat NPHN control/blocking peptide # 1	**SIZE**: 100 ug
Cat. # NPHN11-A	**Rabbit** Anti-Rat NPHN IgG # 1 (aff pure)	**SIZE**: 100 ug

Nephrin is encoded by NPHS1 gene (chromosome 19q13.1), the core protein of the interpodocytes slit diaphragm of kidney glomerulus, these podocytes are highly differentiated with characteristic interdigitating foot processes covering the outer glomerular basement membrane, the space between these foot processes is spanned by a tight junction to provide the large surface area for filtration.

The mutated NPHS1 gene in congenital nephrotic syndrome of Finnish type, a human disease that leads to massive proteinuria in utero and nephrosis at birth. Nephrin is a transmembrane protein of immunoglobulin super family with 8 Ig like domains, identified in human (1241aa), rat (1252aa), and mouse (1256aa). Nephrin is found to be specifically expressed in kidney-slit diaphragm, brain and pancreas, the protein plays a crucial role in development and function of kidney filtration barrier, this barrier is crucial for maintaining the water and electrolyte balance without losing circulating proteins into the urine.

Nephrin interacts with podocin and with CD2AP C-terminal domain, It tends to be a signaling molecule that activates canonical protein kinase cascades, which is initiated by three closely related proteins called NEPH proteins (NEPH1, NEPH2, & NEPH3), The NEPH proteins share a common domain architecture consisting of 5 extracellular Ig domain followed by transmembrane domain, These proteins bind to C-terminal domain of podocin, which interacts with C-terminal of nephrin and greatly enhances nephrin-induced signaling.

FUNCTION: Seems to play a role in the development or function of the kidney glomerular filtration barrier. May anchor the podocyte slit diaphragm to the cytoskeleton.
SUBCELLULAR LOCATION: Cell membrane; Single-pass type I membrane protein (Potential). Note=Located at podocyte slit diaphragm between podocyte foot processes.
SIMILARITY: Belongs to the immunoglobulin superfamily.
Protein name Nephrin [Precursor]
Synonym Renal glomerulus-specific cell adhesion receptor
Gene name Name: Nphs1; Synonyms: Nphn

Source of Antigen and Antibodies

Antigen	20-aa peptide from Rat NPHN1/Nphs1; (protein accession #Q9R044 , refs 1) **Designation (NPHN11-P, control peptide)** conjugated to KLH; Epitope location ~C-terminus, Intracellular domain
Ab Host/type	Rabbit, Polyclonal, purified over antigen-agarose column, Aff pure IgG (cat # NPHN11-A)
2-ab	**Goat Anti-rabbit IgG-HRP** cat # 20320 (AP, biotin, FITC conjugates also available)
-ve control	# 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as –ve control

Form & Storage of Antibodies/Peptide Control

Affinity pure IgG
100 ug/100ul solution lyophilized powder
Supplied in **Buffer**: PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/ml

Control/blocking peptide
100 ug/100 ul solution lyophilized powder
Supplied in Buffer: PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml.

Storage
Short-term: unopened, undiluted liquid vials at -20OC and powder at 4oC or -20oC..

Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.
Shipping: 4oC for solutions and room temp for powder

Recommended Usage

Western Blotting (1-10 ug/ml for affinity pure antibody using ECL technique).

ELISA: Control peptide can be used to coat ELISA plates at 1 ug/ml and detected with antibodies (0.5-1 ug/ml for affinity pure).

Histochemistry & Immunofluorescence: Not tested. We recommend the use of aff pure IgG at 2-20 ug/ml.

Specificity & Cross-reactivity

The Rat NPHN1 1 control peptide is 81% conserved in human and mouse. No significant sequence homology is seen with other related family members Antibody cross-reactivity in various species has not been studied. Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity (see detailed protocol at: www.4adi.com\data\abblock.html).

General References: Yuan H et al (2002) Am.J. Physiol 282, F585-F591, Heli Putaala et al (2001) Human Mol. Gen.10, 1-8; Dorbit B.Donoviel et al (2001) Mol and Celluar Biol 21, 4829-4836, David B. Kershaw et al (1997) JBC 272, 15708-15714. Lorenz Sellin et al (2002) FASEB journal 10 Online (review)

*This product is for In vitro research use only.

Related material available from ADI

Antibodies & Peptides: Nephrin related proteins (NEPH 1-3, Filtrin, Podocin, Podocalyxin).

NPHN11-A wo bsa azide 50906A