ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Sodium Potassium Chloride Cotransporter 2 (NKCC2/BSC1/CCC2/ Slc12a1) Antibodies

Cat. # NKCC21-P	Rat NKCC2 Control/blocking Peptide	**SIZE**: 100 ug
Cat. # NKCC21-S	Rabbit Anti-rat NKCC2 antiserum #1	**SIZE**: 100 ul
Cat. # NKCC21-A	Rabbit Anti-rat NKCC2 IgG #1(aff pure)	**SIZE**: 100 ug

Chloride is a critical component of all living cells. It is also the single most dominant diffusible anion inside of most cells - the others are mostly impermeable organic anions. The cation chloride cotransporters (CCC) protein family is involved in the electroneutral movement of ions across the cell membrane. It includes the thiazide-sensitive Na+-Cl- cotransporters (**NCC or TSC**), the loop diuretics-sensitive Na+-K+-Cl- (NKCC) cotransporters (**NKCC1/CCC1/BSC2 and NKCC2/CCC2/BSC1**), and the K+-Cl--cotransporters (KCC1-4). These co-transporters share a common predicted membrane topology, with 12 TM domains (~500 aa), and long hydrophilic, intracellular N-and C-termini containing regulatory phosphorylation sites. NKCC transport Na, K, and Cl ions into and out of a wide variety of epithelial and non-epithelial cells. The transport process is characterized by electroneutrality (almost always with stoichiometry of 1Na:1K:2Cl) and inhibition by the loop diuretic bumetanide, benzametanide, and furosemide.

Unlike NKCC1, NKCC2 (human 1099 aa, rat/mouse 1095 aa; calculated mol size of ~121 kDa; actual size ~150 kDa; ~45% identity with NKCC1) is most strongly expressed in the kidney (inner and outer stripes of medulla and cortical thick ascending limb), and macula densa. NKCC2 plays a critical role in transcellular absorption of Na+-Cl- by the medullary and cortical TALH, and a secondary role in the paracellular transport of Na-Ca and Mg.

FUNCTION: Electrically silent transporter system. Mediates sodium and chloride reabsorption. Plays a vital role in the regulation of ionic balance and cell volume.
SUBCELLULAR LOCATION: Membrane; Multi-pass membrane protein.
SIMILARITY: Belongs to the SLC12A transporter family.
Protein name Solute carrier family 12 member 1
Synonyms Bumetanide-sensitive sodium-(potassium)-chloride cotransporter 2, Kidney-specific Na-K-Cl symporter
Gene name Name: Slc12a1; Synonyms: Nkcc2

Source of Antigen and Antibodies

Antigen	15-aa peptide of Rat NKCC2/ Slc12a1 (gene accession # P55016); Designated (NKCC21-P or control peptide /blocking peptide) conjugated to KLH; Epitope location ~C-terminus, Cytoplasmic domain
Ab Host/type	Rabbit, Polyclonal unpurified antiserum (#**NKCC21**-S) and IgG, purified over antigen-agarose (Cat # **NKCC21**-A)
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (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

Antiserum (unpurified)
100ul solution lyophilized powder
Supplied in Buffer: 0.05% azide
Reconstitute powder in 100 ul PBS

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 (1:1K-5K for neat serum and 1-10 ug/ml for affinity pure antibody using ECL technique). Full length human NKCC2 is ~150 kDa (1, 2).

ELISA: Control peptide can be used to coat ELISA plates at 1 ug/ml and detected with antibodies (1:10-50K for neat serum and 0.5-1 ug/ml for affinity pure).

Histochemistry & Immunofluorescence: We recommend the use of affinity purified antibody at 2-20 ug/ml in paraformaldehyde fixed sections of tissues (1).

Specificity & Cross-reactivity

The 15 AA rat NKCC21-P control peptide 100% conserved in mouse, 87% in human and rabbit NKCC2. No significant sequence homology is detected with NKCC1 or TSC. Antibody cross-reactivity in various species has not been studied. The **NKCC21-P c**ontrol 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: (1). Gamba G et al (1994) J Biol. Chem. 269, 17713; Simon DB et al (1996) Nature Genet. 13, 183; Mount DB et al (1995) J Am. Soc. Nephrol. 6, 347; Igarashi P et al (1995) Am. J. Physiol. 269, F405;

(2) Citations of ADI's Antibodies (see web site for updated list)

Kreydiyyeh SI, 2004, Cytokine, 26, 1-8 WB
Quaglia NB, 2004, Clin Exp Pharmacol. Physiol. 31,231-236, WB

*This product is for In vitro research use only.

Related antibodies available from ADI
NKCC21-S-A-P 70710A

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