ALPHA DIAGNOSTIC INTERNATIONAL

LIFE TECH Life Technologies™ Your Molecular & Cell Technology Partner

Product Specification Sheet

Sodium Potassium Chloride Cotransporter 1 (NKCC1/BSC2/CCC1) Antibodies

Cat. # NKCC11-P	Rat NKCC1 control/blocking Peptide	**SIZE**: 100 ug
Cat. # NKCC11-S	Rabbit Anti-rat NKCC1 antiserum #1	**SIZE**: 100 ul
Cat. # NKCC11-A	Rabbit Anti-rat NKCC1 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). 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.

NKCC1/BSC2 (human 1212 aa, rat 1203 aa, and mouse 1205 aa; calculated mol. wt of ~115 kDa; actual size is ~145kDa) is strongly expressed in the kidney and many other tissues. In mouse NKCC1 is detected in basolateral membrane of IMCD and in mesangial cells in the glomerulus and basolateral membrane of acinar cells in rat submandubular gland. In the brain, NKCC1 mRNA is most prominent in choroid plexus.

Protein name Na-K-Cl cotransporter
Gene name Name: Slc12a2 ; Synonyms: Nkcc1

Source of Antigen and Antibodies

Antigen	22-aa peptide from **rat NKCC1 (1)/SLC12a2**; (gene accession # MP113986.1) **Designation (#NKCC11-P, control/blocking peptide**) conjugated to KLH; Epitope location ~C-terminus, Cytoplasmic domain
Ab Host/type	Rabbit, Polyclonal unpurified antiserum (#NKCC11-S) and IgG, purified over antigen-agarose (Cat # NKCC11-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, undiluted)
100 ul/vial solution — 50 ul/vial lyophilized powder
contains 0.05% sodium azide
Reconstitute powder 50 ul or 100 ul PBS

Affinity pure IgG
100 ug/100ul solution — 50 ug/50 ul lyophilized powder
Buffer: PBS+0.1% BSA+0.05% azide
Reconstitute powder in PBS at 1mg/ml

Control/blocking peptide
100 ug/100 ul solution — 50 ug/50 ul lyophilized powder
Buffer: PBS pH 7.5, contains 0.05% sodium azide
Reconstitute powder in PBS at 1 mg/ml.

Storage

Short-term: unopened, undiluted liquid vials for less than a week at 4oC.

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 NKCC1 is ~170 kDa (1).

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 22 AA rat NKCC11-P control peptide is 95% conserved in mouse, 86% in human, and 82% in bovine NKCC1. No significant sequence homology is detected with other CCC. Antibody cross-reactivity in various species has not been studied. The NKCC11-P 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:
(1) Moore-Hoon,M.L. et al (1998) Biochim. Biophys. Acta 1373, 261; Delpire,E., et al (1994) J. Biol. Chem. 269, 25677; Payne,J.A., et al (1995) J. Biol. Chem. 270, 17977; Isering P et al (1997) J Biol. Chem. 272, 24556; Mount DB et al (1998) J. Exptl. Biol. 201, 2091-2102 (review).

*This product is for In vitro research use only.

Related material available from ADI

Antibodies CLC1-7 and CLC-K1; KCC1-4; AQP-9 and RUT; NHE1-5; OCT and OAT, AE1-3, and NCX, NaHCO3 (NBC1-3) and NaPi transporters

Western blot Recycling Kit- Reuse blots in Just 5-10 min. (use the same strip for various NKCCs) New formulation will strip antibodies in just a few minutes at room temp. (no boiling or pungent mercaptoethanol).

NKCC11-S-A-P 50201A

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400, Fax: +91-11-42208444
Email: customerservice@lifetechindia.com Website: www.lifetechindia.com