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Solute Transport Carrier 12 (Slc12) Antibodies

Sodium Potassium dependent 2Chloride Co-transporter (NKCC2) or Solute Carrier family 12 (Slc12) antibodies Cat # NKCC2-101AP, FITC-NKCC2, Biotin-NKCC2, P-NKCC2 and PC-NKCC2.

Alternate Nomenclature: Slc12, NKCC2.

Electrically silent Na(+)-K(+)-Cl- transporter systems are present in a wide variety of cells and serve diverse physiological functions. In chloride secretory and absorbing epithelia, these cotransporters provide the chloride entry mechanism crucial for trans-cellular chloride transport. Renal handling of NaCl is a major determinant for homeostasis of salt and extracellular fluid volumes and consequently blood pressure. The kidney specific Na-K-co-transporter (NKCC2) facilitates and mediates the transport of Na and Cl across the luminal membrane of the thick ascending limb of the loop of Henle and the macula densa. NKCC2 mutations and lack of function caused Bartters syndrome and led to salt-wasting in neonates (1). Diuretics like bumetamide, furosemide act on NKCC2. A second physiological function of KCC2 is its involvement in tubuloglomerular feedback to control the renal blood flow. The renal-specific NKCC2 (Na⁺-K⁺-2Cl⁻ co-transporter 2) is regulated by changes in phosphorylation state by metabolic sensing kinase (AMPK) which phosphorylates the serine126 (1). The chloride-sensitive activation of NKCC2 requires the interaction of two serine-threonine kinases, WNK3 and SPAK. WNK3 is positioned upstream of SPAK and appears to be the chloride-sensitive kinase. Elimination of WNK3's unique SPAK-binding motif prevents its activation of NKCC2, as does the mutation of thr 96, 101, and 111 (2). The expression of NKCC2 in the thick ascending loop of Henle is decreased by cGMP mediated by PDE2A (3).

The two major electroneutral sodium-chloride transporters present in the mammalian kidney, the bumetanide-sensitive Na(+)-K(+)-Cl- symporter and thiazide-sensitive Na(+)-Cl- cotransporter, and have characterized. Despite their differing sensitivities to bumetanide and thiazides and their different requirements for potassium, these approximately 115-kDa proteins share significant sequence similarity (approximately 60%) and exhibit a topology featuring 12 potential membrane-spanning helices flanked by long non-hydrophobic domains at the NH2 and COOH termini. The NKCC2 has an apparent molecular weight of around 160kDa in ascending loop of Henle (4).

The NKCC2-selective antibodies were generated against a peptide corresponding to amino acid 33-55 residing on extracytosolic phase. The peptide was covalently modified after synthesis to achieve desired antigenicity before coupling to a carrier protein. The NKCC2-selective antibodies are affinity purified over immobilized antigen based affinity chromatography, and the purified immunoglobulin are stabilized in antibody stabilization buffer for long-term storage. Limited quantities of the antigenic blocking peptide for NKCC2 antibodies is also available (please inquire for availability). *FabGennix Inc.* will conjugate antibodies with enzymes or fluorescent probes (FITC, Cy3, cy5, Rhodamine etc.) as custom service upon request at a nominal cost. For easy identification of NKCC2 on western blots, *FabGennix* also provide Western blot positive controls for NKCC2 in ready-to-use buffer. *FabGennix International Inc.*, has produced a number of antibodies against transporter and solute carriers, please visit www.FabGennix.com for a complete listing.

Catalog #	Description	Host	Cross reactivity	Qty/Price
NKCC2-101AP	Affinity purified Slc12 antibodies	Rabbit	R, M, H, mon	100ug/200ul
FITC-NKCC2	FITC-conjugated NKCC2 antibody	Rabbit	R, M, H, mon	100ug/200ul
P-NKCC2	Antigenic blocking peptide for NKCC2-101AP	n/a	n/a	250ug/100ul
PC-NKCC2	Western blot positive control for NKCC2	n/a	n/a	For 5 appl/inquire

R = rat; M = mouse; H = humans; R = rabbit, monk = monkey

Immunogen: Synthetic peptides (sds tdp phy eet sfg dea qnr lk) taken from NKCC2 protein corresponding to 33-55 amino acid located extracytoplasmic phase. Modified peptide was covalently coupled to a carrier protein by heterobifunctional cross linker for immunogen preparation.
Concentration: NKCC2-101AP = IgG concentration 0.68-0.72mg/ml in antibody stabilization buffer.
Applications: ELISA: Antibody dilution 1:10,000 for ELISA or DOT blot assay. W.B: Antibody dilution 1:500 for WB using PC-NKCC2; IMM: n.d; IHC n.d. The cross species reactivity of this antibody has not been examined in detail.
Reactivity: The antibody a 160kDa NKCC2 protein in PC-NKCC2 samples.
Protocols: Standard protocol for various applications (WB; IMM and IHC) for this antibody can be obtained by calling Technical support line, general information on this antibody is provided with the product specification sheet, and however, *FabGennix Inc.* recommends investigators to optimize conditions.
Form/Storage: The antiserum is supplied in antibody stabilization buffer with preservatives. For long-term storage of antibody, store at -20°C *FabGennix Inc.* does not recommend storage of very dilute antibody solutions unless they are prepared in specially formulated multi-use antibody dilution buffer (Cat # DiluOBuffer). Working solutions of antibodies in DiluOBuffer should be filtered through 0.45um filter after every use for long-term storage.

**Note: Briefly centrifuge to collect liquid before opening the vial, heat the PC-NKCC2 tube in 90oC water bath for 1-2 minutes to dissolve any precipitate before use. This product is "ready-to-use" for electrophoresis. After thawing store at room temperature, Repeated freezing and thawing may result in appearance of higher molecular weight immunoreactive bands.*

Note: Now you can recycle your western blots (nitrocellulose, supported membranes and PVDF membranes) by using our StripOBuffer (Cat FGI-1989). Each stripping is guaranteed to give better signal (up to 8 stripping). No strong pungent smell of reducing agents or heating is required.

References:

1. Scott A. Fraser, et. al., Regulation of the renal-specific Na⁺-K⁺-2Cl⁻ co-transporter NKCC2 by AMP-activated protein kinase (AMPK). *Biochem J.* 2007 July 1; 405 85-93.
2. Ponce-Coria J. et. al., Regulation of NKCC2 by a chloride-sensing mechanism involving the WNK3 and SPAK kinases. *PNAS* 2008 Jun 17;105:8458-63. Epub 2008 Jun 11.
3. Ares GR, Caceres P, Alvarez-Leefmans FJ, Ortiz PA. cGMP decreases surface NKCC2 levels in the thick ascending limb: role of phosphodiesterase 2 (PDE2). *Am J Physiol Renal Physiol.* 2008 Oct;295(4):F877-87. Epub 2008 Aug 6.

** For users who may require large amounts of NKCC2-101AP, please enquire about bulk material discounts.
 This Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.*

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