ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

K+-Cl- Cotransporter-3 (KCC3) Antibodies

Cat. KCC31-S	Rabbit Anti-Human KCC3 Antiserum # 1	**SIZE**: 100 ul
Cat. KCC31-A	Rabbit Anti- Human KCC3 IgG # 1(aff pure)	**SIZE**: 100 ug
Cat. KCC31-P	Human KCC3 Control peptide # 1	**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. Since cytoplasmic electroneutrality is maintained under normal physiological environment, changes in cellular chloride level is accompanied by total cell solute content. The cation chloride cotransporters (**CCC**) protein family is involved in the electroneutral movement of ions across the cell membrane.

The K-Cl cotransporters mediate the coupled movement of K+ and Cl- ions across the cell membranes. This transport process is involved in the regulatory volume decrease in response to cell swelling in red cells and vectorial movement of Cl- ions across the kidney epithelia. The net direction of this transport is out of the cells. At least four isoforms of KCC identified (**KCC1, KCC2, KCC3, and KCC4**) have been identified and functionally characterized. KCC are predicted to have the same protein structure: 12 transmembrane domains with a large extracellular loop with potential N-glycosylation sites, and the cytoplasmic N and C-termini. **KCC3** is ~77% identical to KCC1. Alternative splicing of KCC3 transcripts generates KCC3 with different N-termini (1099 aa and 1150 aa). It is highly expressed in brain, heart, skeletal muscle, and kidney. KCC3 is modulated by VEGF and TNF-alpha.

Source of Antigen and Antibodies

Antigen	19-aa peptide of Human KCC3 ; (Gene Accession #NP005126.1) **Designated (KCC31-P or control peptide or blocking peptide**) conjugated to KLH; **epitope location** ~ N-terminal, Cytoplasmic domain
Ab Host/type	Rabbit, Polyclonal unpurified antiserum (#**KCC31**-S) and IgG, purified over antigen-agarose (Cat # **KCC31**-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 1-10 ug/ml for affinity pure or 1:500:1:000 for neat serum using Chemiluminescence technique.

ELISA (1:10K-1:100K; using 50-100 ng of control peptide/well).

Histochemistry & Immunofluorescence: Not tested. We recommend the use of affinity purified antibody at 2-20 ug/ml in formaldehyde fixed tissue.

Specificity & Cross-reactivity

Human KCC31-P antigenic peptide sequence is found in one of the alternatively spliced form (KCC-3b). KCC31-P is 89% conserved in rat and 78% in mouse KCC-3b. The other form of the human KCC3a (1150 aa; Mount DB et al 1999) does not share this sequence. We have raised another antibody (cat # KCC32-S) using the C-terminal peptide sequence that is found in both forms of KCC3. KCC31-P has no significant sequence homology with other KCCs or CCC. Antibody crossreactivity in various species is not established. We recommend the use of control peptide to establish antibody specificity. 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 see detailed protocol at the web site).).

General References: 1. Hiki K (1999) J Biol. Chem 274, 10661-10667; Mount DB et al (1999) 274, 16355-16362; Mount DB (1998) J Exptl. Biol. 201, 2091; O'Neill WC (1999) Am J. Physiol. 276, C995.

*This product is for in vitro research use only.

Related material available from ADI

Antibodies to KCC1-4, NKCC, NCC, OAT, OCT, AE13, NBC, CLC1-7;

Western Blot recycling kit (Use the same blot to probe with multiple antibodies of KCCs.)

KCC31-S-A-P 71213A

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