ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

K+-Cl- Cotransporter-2 (KCC2) Antibodies

Cat. KCC21-S	Rabbit Anti-Rat KCC2 Antiserum	**SIZE**: 100 ul
Cat. KCC21-A	Rabbit Anti-Rat KCC2 Ig G # 1(affinity pure)	**SIZE**: 100 ug
Cat. KCC21-P	Rat KCC2 control/blocking 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. 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.

Protein name Solute carrier family 12 member 5
Synonyms Electroneutral potassium-chloride cotransporter 2
K-Cl cotransporter 2, Neuronal K-Cl cotransporter
Furosemide-sensitive K-Cl cotransporter, rKCC2
Gene name Name: Slc12a5

FUNCTION: Mediates electroneutral potassium-chloride cotransport in mature neurons. Transport occurs under isotonic conditions, but is activated 20-fold by cell swelling. Important for Cl(-) homeostasis in neurons. Necessary for the ontogenic change in response to gamma-aminobutyric acid (GABA) from depolarization to hyperpolarization during neuronal development.
SUBUNIT: Homomultimer and heteromultimer with other K-Cl cotransporters (By similarity).
SUBCELLULAR LOCATION: Multi-pass membrane protein.
TISSUE SPECIFICITY: Highly expressed in brain. Not detected in other tissues. Highly expressed in pyramidal neurons and in neurons throughout the cortex, hippocampus, the granular layer of the cerebellum and in groups of neurons throughout the brainstem. Barely detectable in dorsal-root ganglions.
DEVELOPMENTAL STAGE: Detected in thalamus, but not in hippocampus and neocortex at E20. At birth barely detectable in hippocampus. Expression increases steeply from day 5 to day 9 and then stabilizes at adult levels.
SIMILARITY: Belongs to the SLC12A transporter family.

Source of Antigen and Antibodies

Antigen	18aa peptide of Rat KCC2 ; (Gene Accession #Q63633, 1116-aa) **Designated (KCC21-P or control peptide). conjugated to KLH; epitope location ~ N-terminal, Cytoplasmic domain**
Ab Host/type	Rabbit, polyclonal Unpurified antiserum (cat #KCC21-S) Aff pure IgG (**cat #KCC21-A**) purified over antigen-agarose column
-ve control	# 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as –ve control
2-ab	**Goat Anti-rabbit IgG-HRP** cat # 20320 (AP, biotin, FITC conjugates also available)

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

Rat KCC21-P sequence is 94% conserved in mouse and human. No significant sequence homology is seen with other KCCs or CCC. 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.

General References: 1. Payne JA et al (1996) J. Biol. Chem. 271, 16245; Gillen CM et al (1996) J. Biol. Chem. 271, 16237; Haap S et al (1998) gene Accession # AJ011033; Mount DB et al (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, OAT1-4, OCT, AE13, NBCs, CLC1-7;
KCC21-S-A-P 71213A

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