ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Chloride Channel-5 (CLC-5 or CLCN5) Antibodies

Cat. # CLC51-PRat CLC-5 Control Peptide **SIZE**: 100 ug

Cat. # CLC51-SRabbit Anti-rat CLC-5 antiserum #1 **SIZE**: 100 ul

Cat. # CLC51-ARabbit Anti-rat CLC-5 Ig G #1 (affinity pure) **SIZE**: 100 ug

Chloride is a critical component of all living cells. Voltage-gated chloride channels regulate cellular traffic of chloride ion. The chloride channels (CIC or CLC) performs several functions including the regulation of cell volume, membrane potential stabilization, signal transduction, and transepithelial transport. Mutations in CIC genes have been linked with several human diseases including myotonias (Thomsen's disease), cystic fibrosis, Bartters syndrome type III, Dent's disease, and X-linked recessive nephrolithiasis. In mammals, CLC proteins form a superfamily of at least 9 different genes (CLC1-7 also known as CLCN1-7 and CLK1-2 or CLCKa and CLCKb). Additional forms of these proteins are obtained by alternative splicing. All CLC proteins (~700-1000 aa) are predicted to contain 10 (possibly 12) transmembrane domains. Except CLC-1 and CLC-K1/K2 that are specific for kidney, most other CLC are widely distributed in various tissues.

Rat CLC-5 is 746 aa membrane protein (human CLC-5 746) (1). CLC-5 is abundant in kidney moderate levels are found in the brain, lung, and liver. Mutations or deletions in CLC-5 have been linked with Dent's diseases (excessive urinary calcium, urinary low mol wt proteinuria, and calcium kidney stone), and four related X-linked syndromes of hypercalciuric nephrolithiasis.

Source of Antigen and Antibodies

Antigen	13aa peptide of rat CLC5; **Designated (CLC51-P or control peptide).** epitope location ~ C-terminus
Ab Host/type	Rabbit, polyclonal Unpurified antiserum (cat #CLC51-S) Aff pure IgG1 (**cat #CLC51-A**) purified over antigen-agarose column
2-ab	**Goat Anti-rabbit IgG-HRP** cat # 20320 (AP, biotin, FITC conjugates also available)
-ve control IgG	# 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 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). Antibodies made to various CLC-5 peptides have been shown to detect a protein of approx. 80 kDa in kidney (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 1-20 ug/ml in paraformaldehyde fixed sections of tissues. Antibodies to CLC-5 peptides have been used to localize CLC-5 in renal proximal tubule cells (2).

Specificity & Cross-reactivity

The 13 AA rat CLC51-P control peptide is 100% conserved in mouse, pig, rabbit, 84% in guinea pig and 92% in human CLC-5. No significant sequence homology is detected with other CLCs or other proteins. Actual cross-reactivity of antibodies in various species has not been studied. There is a very high degree of protein homology among CLC-3, CLC-4, and CLC-5. However, CLC51-P peptide has very little sequence homology with CLC-3, and CLC-4. Anti-rat CLC41 antibodies were checked against the corresponding CLC-3 (Cat # CLC31-P) and CLC-4 (Cat # CLC41-P). These peptides are also available to confirm specificity of antibodies. 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 the web site).).

General References: Steinmeyer K et al (1995) J Biol. Chem. 270, 31172-31177; Sakomoto H et al (1996) J Biol. Chem. 271, 10210-10216; Fisher SE et al (1995) Genomics 29, 598-606; Lloyd SE et al (1994) Hum Mol. Genet. 3, 2053-2059; Lloyd SE et al (1996) nature 379, 445, Gunther W et al (1998) PNAS 95, 8075-8080.

*This product is for In vitro research use only.

Related material available from ADI

Antibodies CLC1-7 and CLC-K1; KCCL1-3; AQP-9 and RUT; OCT and OAT, AE-3, and NACX

CLC51-S-A-P 71218A

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