ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

K+-dependent, Na Ca Exchanger 1 (NCKX1) Antibodies

Cat. # NCKX12-P	Rat NCKX1 control/blocking Peptide #2	**SIZE**: 100 ug
Cat. # NCKX12-S	Rabbit Anti-Rat NCKX1 antiserum #2	**SIZE**: 100 ul
Cat. # NCKX12-A	Rabbit Anti-Rat NCKX1 IgG #2 (aff pure)	**SIZE**: 100 ug

Ca2+ plays a critical role in intracellular signaling. Intracellular Ca2+ levels are tightly controlled by continuous removal of Ca2+ via ATP-driven **Ca2+ pump** in the endoplasmic reticulum and plasma membrane, and the **Na+/Ca2+ exchangers (NCX)**, in the plasma membrane. NCX can move Ca2+ either into or out of cells, depending on the net Na+, Ca2+, and K+ gradient across the membrane. In most cells, 3 Na+ are exchanged for 1 Ca2+. Several genes code for the 3 **NCX (NCX1, NCX2, and NCX3),** and three in the **NCKX family (NCKX1, NCKX2, NCKX3**). NCX share significant sequence homology (~70%), display 11 TM domains, a large central, intracellular hydrophilic regulatory loop between TM5 and 6, extracellular N-terminus and cytoplasmic C-terminus. The N-terminal signal peptide is cleaved off from the mature exchanger protein.

In vertebrate photoreceptors, some neurons, and certain other cells, 3 K+ are transported in the same direction as Ca^{2+}, with coupling ratio of 4 Na+ to 1 Ca^{2+} plus 1 K+ by new class of exchangers called **NCKX for K+-dependent Na/Ca+2 exchangers**. NCKX share the general topology and functional properties of NCX proteins. Rod exchangers also contains a large hydrophilic segment at the its N-terminus that is not found in NCX. **NCKX1** (human 1099/1081 aa, bovine 1199 aa) is expressed in retinal rod cells. **NCKX2** (rat 670 aa) shares 80% identity with NCKX1. The two hydrophilic loops are also much shorter in NKKX2 than in NCKX1, accounting largely for the difference between the size of the two proteins. NCKX2 is expressed in various regions of the brain (striatum, parietal cortex, cerebellum, hippocampus, and thalamus). A third member of NCKX family, namely **NCKX3** (rat 624 aa, mouse 595, and human 644/625) has been cloned and characterized. Human NCKX3 is most abundant in the brain (thalamic nuclei, hippocampal CA1 neurons, layer IV of the cerebral cortex). It is also expressed at lower levels in aorta, uterus, and intestine.

FUNCTION: Critical component of the visual transduction cascade, controlling the calcium concentration of outer segments during light and darkness.
SUBCELLULAR LOCATION: Membrane; Multi-pass membrane protein.
SIMILARITY: Belongs to the sodium/potassium/calcium exchanger family. SLC24A subfamily.

Protein name Sodium/potassium/calcium exchanger 1
Synonyms Na(+)/K(+)/Ca(2+)-exchange protein 1
Retinal rod Na-Ca+K exchanger
Gene name Name: Slc24a1
Synonyms: Nckx1

Source of Antigen and Antibodies

Antigen	15aa peptide of Rat (gene accession # Q9QZM6) **NCKX1**; **Designated (NCKX12-P or control peptide). conjugated to KLH**~N-terminal, Extracellular
Ab Host/type	Rabbit, polyclonal; Unpurified antiserum (cat #NCKX12-S) Aff pure IgG (**cat #NCKX12-A**)
2-ab	**Goat Anti-rabbit IgG-HRP** cat # 20320 (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 (1:1K-5K for neat serum and 1-10 ug/ml for affinity pure antibody using ECL technique).

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: Not tested. We recommend the use of affinity purified antibody at 5-20 ug/ml in paraformaldehyde fixed sections of tissues.

Specificity & Cross-reactivity

The rat NCKX12-P control peptide is 100% conserved in mouse. It has no appreciable homology with human NCKX1. No significant sequence homology is detected with rat NCKX2 or –3 other NCX. 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 (see detailed protocol at: www.4adi.com\data\abblock.html).

General References:

(1). Poon S et al (1999) Am. J. Physiol. 278, C651-C660; Tucker JE et al (1998) Invest. Opthalmol. 39, 435; Walter MA et al (1998) Human Genet. 103, 411; ; Reilander H et al (1992) EMBO J. 11, 1689; Kim TSY et al (1998) J Biol. Chem. 273, 16561-16567.

Citation of ADI's antibodies for NCX1: Okafor M, 2003, Sodium–calcium exchange influences the response to endothelin-1 in lens epithelium, Cell Calcium May 2003 in press

*This product is for In vitro research use only.

Related material available from ADI

Antibodies NCX1-3/NCKX1-3, CLC1-7/CLC-K1; KCC1-3; AQP1-9 and RUT; OCT1-3, OAT1-3, AE1-4, and NCKX1-3, NaPi-1-III, and NBC1-5 1-3, NHE1-5

(NCKX12-S-A-P . Rev. 40203S)

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