ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Exchange Inhibitor Peptide (XIP) Antibodies

Cat. # XIP11-P	Rat XIP Control Peptide	**SIZE**: 100 ug
Cat. # XIP11-S	Rabbit Anti-rat XIP antiserum	**SIZE**: 100 ul
Cat. # XIP11-A	Rabbit Anti-rat XIP IgG (aff pure)	**SIZE**: 100 ug

Ca2+ plays a critical role in intracellular signaling. Intracellular Ca2+ levels are tightly controlled by continuos removal of Ca2+ via ATP-driven **Ca2+ pump** in the endoplasmic reticulum and plasma membrane, and Ca2+ transport system, 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+. In mammals, at least 5 distinct genes code for the exchangers: Three **NCX (NCX1, NCX2, and NCX3),** and two in the **NCKX family (NCKX1 and NCKX2**). 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.

NCX contains a highly basic region in the large hydrophilic, intracellular loop called **XIP (Exchange inhibitory peptide**; RRLLFYKYVYKRYRAGKQRG 20 aa), that inhibits Na-Ca+ exchange in cardiac sarcolemmal vesicles and in other cells. Little or no sequence identity is found between the NCX and the Ca-pump. However, XIP also inhibits the Ca pumps with more or less same efficiency as **C28R2** peptide sequence (LRRGQILWFRGLNRIQTQIRVVKAFRSS, 28 aa) corresponding to the autoinhibitory domain of the Ca-pump.

Source of Antigen and Antibodies

Antigen	20-aa peptide from **rat XIP (1)**; **Designation (XIP11-P, control or blocking peptide**) conjugated to KLH; epitope location ~ N-terminus
Ab Host/type	Rabbit, Polyclonal unpurified antiserum (#**XIP11**-S) and IgG, purified over antigen-agarose (Cat # **XIP11**-A)
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (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).

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 20 AA rat XIP is 100% conserved in mouse, rat, human, rabbit, dog, cat, frog XIP. It is 70% conserved in rat NCX2 and NCX3. Antibody cross-reactivity in various species has not been studied. The **XIP11-P c**ontrol 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:

(1). Hale CC et al (1997) BBRC 236, 113; Xu W et al (1997) Arch. Biochem. Biophys. 341, 273; Low W et al (1993) FEBS Lett. 316, 63; Furman I et al (1993) FEBS Lett. 319, 105; Lee SL et al (1994) J Biol. Chem. 269, 14849; Nicoll DA et al (1996) J Biol. Chem. 271, 24914;; Blaustein MP and Lederer J (1999) Physiol Rev. 79, 763-854 (review).

Citations of ADI's Antibodies for XIP:

Zhong, N, 2001, J. Neurosci. 21: 9598-9607, Roles for Mitochondrial and Reverse Mode Na+/Ca2+ Exchange and the Plasmalemma Ca2+ ATPase in Post-Tetanic Potentiation at Crayfish Neuromuscular Junctions.

*This product is for In vitro research use only.

Related material available from ADI

Antibodies CLC1-7 and CLC-K1; KCCL1-3; AQP1-9 and RUT; OCT/OCTN1-3, OAT1-3, OATK1/K2, AE1-3, and NCKX1-3, NaPi and NaHCO3 transporters 1-3, NHE1-5

XIP11-S-A-P 71209A

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