ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Plasma Membrane Ca+-ATPase (PMCA)/Ca+-Pump Antibodies

Cat. # PMCA31-M Mouse Monoclonal Anti-Human PMCA ascites **SIZE**: 100 ul

FORM: Soln Lyophilized.

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 Ca-pump.

Ca+-pump is a Mg+-dependent enzyme that catalyzes the hydrolysis of ATP with the transport of Ca+. Plasma membrane Ca+-ATPase (calcium pump) exists in several isoforms (human isoforms 1, 1084 aa; 1b, 1220 aa; from 2, 198/1243 aa; 3a/b 1173/1220 aa; 4, 1205 aa) and numerous sub-isoforms have been identified in various species. Ca+-pump displays 10 TM domains, with cytoplasmic N and C-termini. C28R2 is located in within the cytoplasmic, C-terminal domain of Ca+-ATPases.

Source of Antigen and Antibodies

Purified human erythrocyte Ca+-ATPases was used to immunize mice (1). Resulting clone designated PMCA31-M secrete IgG2a antibody. Antibody is supplied as ascites produced in mice. The epitope of this antibody has been mapped to 724-783 aa (hinge region) of Ca+-ATPases.

Recommended 2-antibodies: **Goat Anti-mouse IgG-HRP conjugate** Cat # 40320 (AP, biotin, FITC conjugates also available)

Form & Storage

The antibody is supplied as neat ascites (100 ul soln or lyophilized) containing 0.1% sodium azide as preservative. **Lyophilized products** should be reconstituted in 100-ul water and gently mixed for 15 min at room temp. All peptide/antibody received in solution or reconstituted from lyophilized vials should be stored frozen at -20°C or below in suitable aliquots. It is not recommended to store diluted solutions.

Recommended Usage

Western Blotting Dilute neat ascites 1:100-1:500 for Western using ECL technique. Antibody reacts with 138 kDa human erythrocyte Ca+-ATPase (1, 2). It may also recognize some other aggregated band or proteolytic products. Antibody crossreactivity is seen with Ca+-pump of basolateral membrane of convoluted distal tubules of kidney, hepatocytes plasma membrane, intestine, placenta, endometrium, choroid plexus, Purkinje cells and he oviduct tissue (1, 2).

ELISA: Dilute antibody at 1:1K-5K for neat ascites.

Histochemistry & Immunofluorescence: This antibody can be used to localize Ca+-ATPases using formalin-fixed, paraffin-embedded tissues sections (1, 2). In human kidney, specific staining is seen along basolateral membrane of the distal convoluted tubules.

Specificity & Cross-reactivity

Anti-human erythrocyte Ca+-ATPases PMCA31-M crossreacts with human, chimpanzee, baboon, monkey, dog, rabbit, cat, rat, mouse, chicken, eel, plants, and parasites (2).

General References:

(1). Borke JL et al (1987) J Clin Invest. 80, 1225; Borke Jl et al (1989) Am. J. Physiol. 257, F842

(2). Borke Jl et al (1989) Brain Res. 489, 355; Papp B et al (1989) J Biol. Chem. 264, 4577; Wasserman RH et al (1991) Histochem. 96, 413; Kessler F et al (1990) J Biol. Chem. 265, 16012; Borke JL et al (1989) Am J. Physiolo. 257, C341; De Talamoni NT et al (1993) PNAS 90, 11949; Magocsi M et al (1991) BBA 1063, 7; Adamo HP et al (1992) Biochem J. 285, 791; Adamo HP et al (1992) J Biol. Chem. 267, 14244; Blaustein MP and Lederer J (1999) Physiol Rev. 79, 763-854 (review).

*This product is for In vitro research use only.

PMCA31-M 50426A

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