3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

MONOCLONAL Anti-Ca^{2+}-ATPase, HUMAN ERYTHROCYTE
CLONE 5F10
Mouse Ascites Fluid

Product No. **A 7952**

Product Description

Monoclonal Anti-Ca^{2+}-ATPase, Human Erythrocyte, (mouse IgG2a isotype) is derived from the 5F10 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with purified human erythrocyte Ca^{2+}-ATPase.[1,2] The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Ca^{2+}-ATPase, Human Erythrocyte , reacts specifically with human erythrocyte Ca^{2+} pump (Ca^{2+}-ATPase, 138 kDa) in immunoblotting.[2-12] It may recognize additional bands at approx. 215, 160-180, 125, 95-107, 72 and 27 kDa, that may represent aggregation or proteolysis (dimers or combination of monomer with proteolytic products) of the pump.[2,5-9] The product does not significantly affect the Ca^{2+} pump activity.[2,4,12] The reactivity of the antibody has been mapped to an epitope within amino acid residues 724-783, the region which is proposed to be the "hinge domain" of the Ca^{2+}-ATPase molecule. [12] The product recognizes the Ca^{2+} pump of other sources (e.g., basolateral membrane of distal convoluted tubules of the kidney,[1-3] hepatocyte plasma membranes,[6] intestine,[9] placenta,[7] endometrium,[10] choroid plexus,[3] Purkinje cells,[8] hen oviductal tissue [5]) in immunohistochemistry and immunoblotting. In human kidney, specific staining is found along the basolateral membrane of the distal convoluted tubules, while glomeruli and other segments of the nephron do not stain. The antibody may be used for immunohistochemical labeling of formalin-fixed, paraffin-embedded tissue sections,[2,3,5-7,9] as well as for ELISA,[2] immunoaffinity purification of the Ca^{2+} pump[6] and immunoprecipitation. Cross-reactivity has been reported with human,[2-4,7,10-12] chimpanzee,[2] baboon,[2] monkey,[2] dog,[2] rabbit, [2] cat,[3] rat,[2,3,5-10] chicken,[5,8] eel, plants and parasites.

Intracellular Ca^{2+} levels are maintained in a number of tissues, partly through the activity of the plasma membrane Ca^{2+} pump. Ca $^{2+}$-ATPase extrudes Ca^{2+} from the cytoplasm against a steep concentration gradient and is essential in maintaining intracellular Ca^{2+} homeostasis.[13] Ca $^{2+}$-ATPase (Ca^{2+} Mg^{2+} adenosine triphosphatase) is a P-type ion-motive ATPase, i.e., it forms an aspartyl phosphate during the reaction cycle and is inhibited by low concentrations of vanadate.[6,13] Ca^{2+} pumps in a number of systems have been shown to share several characteristics. For example, these enzymes have a molecular mass of approx. 140 kDa and form Ca^{2+}-dependent phosphorylated intermediates. In addition, these enzymes bind calmodulin and transport Ca^{2+} at a higher rate in the presence of calmodulin than in its absence.[2,13] Four isoforms (PMCA1-4), encoded by a multigene family, have up to 85-90% similarity in amino acid sequence. In addition, several alternatively spliced products are known to be expressed differentially in various cells.[14] The isoforms differ essentially in the C-terminal regulatory portion. The latter contains a calmodulin binding domain. The regulation by calmodulin is a distinctive property of the ATPase and has permitted its isolation using calmodulin affinity chromatography.[6] Biochemical and immunohistochemical studies at the light microscopic level have shown that the plasma membrane domains of several Ca^{2+} transporting epithelia are not homogenous in the content of Ca^{2+} pump; some seem to have a greater amount of the protein than others.[14] Several domains have been reported to contain abundant amounts of Ca^{2+} pump, including the basolateral surface of small intestinal and renal distal convoluted tubular epithelial cells,[1,2] the sinusoidal surface of hepatocytes,[6] the apical surface of oviduct epithelial cells of the laying hen,[5] and the CSF-facing membranes of choroid plexus cells from rat, cat and man.[3] Monoclonal antibody reacting specifically with Ca^{2+}-ATPase of various cellular sources may be used to study the mechanisms involved in Ca^{2+} transport.

Reagents
The product is provided as ascites fluid with 0.1% sodium azide as a preservative.

Precautions and Disclaimer
Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Uses
Monoclonal Anti-Ca^{2+}-ATPase, Human Erythrocyte, may be used for the localization of Ca^{2+}-ATPase using various immunochemical assays including ELISA, immunoblot, immunohistochemistry, immunocyto-chemistry, immunoprecipitation and immuno-affinity chromatography.

Titer: 1:100
The antibody titer was determined by immunoblot using extracts of human erythrocyte ghosts.

In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

Storage
For continuous use, store at 2-8 °C for up to one month. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

References

1. Borke, J., et al., J. Clin. Invest., **80**, 1225 (1987).
2. Borke, J., et al., Am. J. Physiol., **257**, F842 (1989).
3. Borke, J., et al., Brain Res., **489**, 355 (1989).
4. Papp, B., et al., J. Biol. Chem., **264**, 4577 (1989).
5. Wasserman, R., et al., Histochemistry, **96**, 413 (1991).
6. Kessler, F., et al., J. Biol. Chem., **265**, 16012 (1990).
7. Borke, J., et al., Am. J. Physiol., **257**, C341 (1989).
8. De Talamoni, N., et al., Proc. Natl. Acad. Sci. USA, **90**, 11949 (1993).
9. Borke, J., et al., Pflugers Arch., **417**, 120 (1990).
10. Magocsi, M., and Penniston, J., Biochim. Biophys. Acta, **1063**, 7 (1991).
11. Adamo, H., et al., Biochem. J., **285**, 791 (1992).
12. Adamo, H., et al., J. Biol. Chem., **267**, 14244 (1992).
13. Carafoli, E., Physiol. Rev., **71**, 129 (1991).
14. Fujimoto, T., J. Cell Biol., **120**, 1147 (1993).

10/98