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Product Information

MONOCLONAL ANTI- Na^+/K^+ ATPASE ($\alpha 3$ SUBUNIT), CLONE XVIF9-G10 Mouse Ascites Fluid

Product Number **A-273**

Product Description

Monoclonal Anti- Na^+/K^+ -ATPase ($\alpha 3$ subunit) (mouse IgG1 isotype) is produced in mouse. Canine cardiac microsomes were used as immunogen.

Monoclonal Anti- Na^+/K^+ -ATPase ($\alpha 3$ subunit) reacts specifically with Na^+/K^+ ATPase $\alpha 3$ subunit. The antibody may be used to localize and detect Na^+/K^+ ATPase $\alpha 3$ subunit (approx. 110 kDa) by immunoblotting and immunohistochemistry. By immunoblotting, reactivity is observed in human, monkey, sheep, canine, rabbit, guinea pig and rat tissues in which the $\alpha 3$ subunit is expressed. By immunohistochemistry, staining is consistent with the plasma membrane localization of Na^+/K^+ ATPase.

The Na^+/K^+ -ATPase is an integral membrane enzyme found in all cells of higher organisms and is responsible for the ATP-dependent transport of Na^+ and K^+ across the cell membrane. This membrane-bound enzyme is related to a number of other ATPases including the sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) and the plasma membrane Ca^{2+} -ATPase (PMCA). The Na^+/K^+ -ATPase consists of a large, multipass, trans-membrane catalytic subunit, termed the α subunit, and an associated smaller glycoprotein, termed the β subunit. Studies indicate that there are three isoforms of the α subunit ($\alpha 1$, $\alpha 2$, $\alpha 3$) and two isoforms of the β subunit ($\beta 1$ and $\beta 2$) which are encoded by two multigene families.

The different isoforms of the Na^+/K^+ -ATPase exhibit a tissue-specific and developmental pattern of expression. The $\alpha 1$ and β mRNAs are present in all cell types examined whereas the $\alpha 2$ and $\alpha 3$ mRNAs exhibit a more restricted pattern of cell-specific expression. The $\alpha 3$ subunit has been found in neuronal and to a lesser extent skeletal and cardiac muscle and lung and stomach tissues.

Reagents

Monoclonal Anti- Na^+/K^+ -ATPase ($\alpha 3$ subunit) antibody is supplied as ascites diluted in phosphate buffered saline (PBS) containing 0.05% sodium azide as a preservative.

Precautions and Disclaimer

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

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze 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. Working dilution samples should be discarded if not used within 12 hours.

Procedure

Immunoblotting

1. Use 10% SDS-PAGE gel to separate proteins. Transfer to nitrocellulose.
2. Incubate membrane in blocking solution which contains 0.5% Tween 20 in Tris Buffered Saline (TBS) for 1 hour at room temperature.
3. Wash three times for 15 minutes each time with TBS and Tween 20 (0.5%) at room temperature. (WASH)
4. Incubate membrane for 1 hour at room temperature with anti- Na^+/K^+ ATPase (Product No. A-273) diluted to 1 $\mu\text{g}/\text{ml}$ in TBS with 0.5% Tween 20.
5. WASH.

6. Incubate membrane for 1 hour at room temperature with Goat anti-mouse IgG-Peroxidase Conjugate (Cat. No. A9917) diluted with 0.5 % Tween 20 in TBS.
7. WASH.
8. React membrane with diaminobenzidine tetrahydrochloride (DAB) in H₂O₂ to effect. Stop reaction by rinsing with dH₂O

Note: The procedure listed above is intended only as a guide. The results that you obtain may vary depending on experimental conditions and technique. No warranty or guarantee of performance of the above procedure is made or implied. Use good laboratory practices, handle all materials carefully.

Product Profile

Suggested working dilution is at least 1 µg/ml by immunoblotting and 3 µg/ml by immunohistochemistry. However, optimal working concentration should be determined by serial dilutions.

References

1. Basavappa, S., et al., J. Cell Physiol., **174**, 145-153 (1998).
2. Kawai, H., et al., J. Neurochem., **69**, 330-339 (1997).
3. Zahler, R., et al., Circ. Res., **78**, 870-879 (1996).

RYC/LPG 3/03

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