

Product Information

Monoclonal Anti-Ryanodine Receptor

Clone C3-33

Diluted Mouse Ascites Fluid

Product Number **R-128**

Product Description

Monoclonal Anti-Ryanodine Receptor (IgG1 isotype) is produced in mice. Purified canine cardiac ryanodine receptor was used as immunogen. Mouse ascites was affinity purified using the antigen.

Monoclonal Anti-Ryanodine Receptor reacts strongly with RyR-2 (expressed predominantly in the heart muscle, but also found in stomach, endothelial cells and diffuse areas of the brain; also known as the β isoform). It reacts weakly with RyR-1 (expressed predominantly in skeletal muscle and areas of the brain; also known as the α isoform). The antibody cross-reacts with ryanodine receptor in cardiac muscle of canine, rat, finch and pigeon; in visceral smooth muscle of toad; and in rat brain. It reacts with skeletal muscle of fish and the β isoform of frog, but only weakly with skeletal muscle in rabbit and the α isoform of frog. The antibody may be used to localize and detect the RyR (approx. 565 kDa) by immunoblotting, immunoprecipitation and immunohistochemistry. In immunoblotting, the antibody detects a 565 kDa band. However, in non-mammalian vertebrates, a doublet is seen at 565 kDa representing the α and β isoforms. In imm-uno histochemistry, it localizes the sarcoplasmic/endoplasmic reticulum calcium pump (SERCA) in rat brain.

The ryanodine receptor (RyR) is the channel responsible for the release of Ca^{2+} from the sarcoplasmic reticulum (SR) in muscle cells and also plays a role in Ca^{2+} regulation in non-muscle cells. The RyR exists as a homotetramer and is predicted to have a short cytoplasmic C-terminus and 4-10 transmembrane domains; the remainder of the protein, termed the "foot" region is located in the cytoplasm between the T-tubule and the SR.

The mammalian RyR is the product of three different genes: RyR-1, which is expressed predominantly in skeletal muscle and areas of the brain, RyR-2, which is expressed predominantly in the heart muscle but also found in the stomach, endothelial cells and diffuse areas of the brain, and RyR-3 which is found in smooth muscle and the brain (striatum, thalamus and hippocampus).

Reagents

Monoclonal Anti-Ryanodine Receptor is supplied as diluted ascites fluid containing 0.05% sodium azide.

Precautions and Disclaimer

Sodium Azide is considered highly toxic and highly reactive under certain conditions. Read the Material Safety Data Sheet carefully before use.

Storage/Stability

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

Procedure

Immunoblotting

1. Use 3-12% SDS-PAGE gradient gel to separate proteins. Transfer to PVDF membrane.
2. Incubate membrane in blocking solution which contains 5% non-fat dried milk and 0.1% Tween 20 in Phosphate Buffered Saline (PBS) for 1 hour at room temperature.

3. Wash three times for 10 minutes each time with PBS and Tween 20 (0.1%) at room temperature. (WASH)
4. Incubate membrane overnight at room temperature with anti-Ryanodine Receptor (Cat No. R-128) diluted 1 µg/ml with 1% non-fat dried milk and 0.1% Tween 20 in PBS.
5. WASH.
6. Incubate membrane for 1 hour at room temperature with Goat anti-mouse IgG-Peroxidase Conjugate (Cat. No. A 9917) diluted with 1% non-fat dried milk and 0.1% Tween 20 in PBS.
7. WASH.
8. React membrane with diaminobenzidine tetrahydrochloride (DAB) in 0.003% 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

Recommended starting titer is 1 µg/ml for immunoblotting and immunohistology. However, optimal working concentration should be determined by serial dilutions.

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

References

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3. McPherson, P.S. et al. J. Biol. Chem. **268**, 19785-19790 (1993).
4. Lai, F.A. et al. Am. J. Physiol. **263**, C365-C372 (1992).

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