SIGMA CHEMICAL COMPANY

Product No. C-7055
Lot 086H4810

Monoclonal Anti-Calmodulin

Mouse Ascites Fluids
Clones 2D1+1F11+6D4

Monoclonal Anti-Calmodulin (mouse IgG1 isotypes) is a mixture of 3 antibodies derived from the hybridomas 2D1, 1F11 and 6D4. Each hybridoma was produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with calmodulin purified from *Dictyostelium discoideum* and conjugated to KLH. Each isotype is determined using the Sigma ImmunoType™ Kit (Sigma Stock No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The product is provided as ascites fluid with 0.1% sodium azide (see MSDS)* as preservative.

Specificity

Monoclonal Anti-Calmodulin[1] recognizes epitopes found in native and SDS-denatured calmodulin of the eukaryotic microorganism *Dictyostelium discoideum*, bovine, rat and chicken brain, applying the immunoblotting technique. The antibody mixture labels the 17 kD band of calmodulin, although in extremely sensitive immunoblotting conditions a faintly stained band that migrates more rapidly than calmodulin and represents trace amounts of calmodulin degradation products, may also be stained. The product is directed against several epitopes on the calmodulin molecule. The antibody mixture does not cross-react with members of the EF-hand motif family: Parvalbumin (rabbit, frog), Troponin (porcine, rabbit, bovine, chicken), S-100 and Myosin Light Chain Kinase (chicken).

Description

Calmodulin[2] is a highly conserved 17 kD calcium-binding protein found in all eukaryotic cells. It is a multifunctional, ubiquitous molecule which can bind up to 4 calcium ions, regulating and mediating a wide variety of biochemical processes. Calmodulin belongs to a family of structurally homologous Ca^{2+}-binding proteins, which includes troponin C, Parvalbumin and S-100. Antibodies reacting with calmodulin are useful tools for the immunohistochemical localization of calmodulin in human and animal tissue sections.[3] Because it is small, acidic and highly conserved, it is an extremely poor antigen.[4]

Uses

Monoclonal Anti-Calmodulin may be used for the localization of calmodulin and the study of interactions with biologically active compounds using various immunochemical assays such as ELISA, immunoblot, dot blot and immunocytochemistry.

Titer: 1:100

The antibody titer was determined by immunoblotting using bovine brain calmodulin preparation.

Notes

1. For sensitive detection of calmodulin bound to membranes, follow the attached procedure for immunoblotting of calmodulin.
2. In order to obtain best results in different techniques and preparations, it is recommended to determine optimal working dilutions by titration assay.

Storage

For continuous use, store at 2-8°C. 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.

* 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.

Procedure for Immunoblotting of Calmodulin[1]

Materials

1. Membrane: Immobilon PVDF transfer membrane (Millipore).
2. KP Buffer: 25 mM KH_2PO_4/K_2HPO_4 buffer, pH 7.0.
3. Absolute methanol.
4. Fixation Buffer: 0.2% (v/v) glutaraldehyde, freshly prepared in KP Buffer.
5. TBS (Tris Buffered Saline): 20 mM Tris, 500 mM NaCl, pH 7.5.
6. Blocking Buffer: 5% (w/v) BSA in TBS (use heat-inactivated globulin-free BSA, e.g., Sigma Product No. A7638).
7. TBS-BSA: 1% BSA in TBS.
8. Washing Buffer: 1% BSA, 0.05% Tween-20 (w/v) in TBS.
9. Primary Antibody: Monoclonal Mouse Anti-Calmodulin.
10. Secondary Antibody: Mouse ExtrAvidin Peroxidase Staining Kit (Sigma Stock No. EXTRA-2, consisting of biotinylated antibody and ExtrAvidin-Peroxidase).
11. Enzyme Substrate: Prepare the following stocks and store at 4°C: Solution A: AEC (3-Amino-9-Ethylcarbazole, Sigma Product No. A-6926), 20 mg in 2.5 ml DMF (Dimethylformamide).
    Solution B: 0.05 M Acetate Buffer pH 5.0.
    Solution C: 3% H_20_2
    Just before use, mix 0.2 ml of Solution A with 3.8 ml Solution B. Add 0.02 ml Solution C. Mix.

Procedure

Notes:

a. Membranes should be kept wet throughout the entire procedure.
b. All incubations and rinses are with gentle agitation.

1. Soak SDS polyacrylamide gel for 15 min. in KP buffer.
2. Pre-wet membrane briefly in absolute methanol. Immerse the membrane in water for 2-3 min. to elute the methanol. Rinse for 15 min. in KP Buffer.
3. Conduct transfer in KP buffer (e.g., 20 V overnight at 4°C).
4. Incubate the membrane for 45 min. at RT in Fixation Buffer.
5. Rinse in KP buffer and block in Blocking Buffer, overnight at 4°C or for 1 hr. at 37°C.
6. Rinse once, 10 min. at RT using TBS.
7. Incubate with Primary Antibody, diluted in TBS-BSA for 1 hr. at 37°C. Rinse 3X, 10 min. each at RT using Washing Buffer.
8. Incubate with Secondary Antibody, diluted in Washing Buffer.
9. Incubate with ExtrAvidin-Peroxidase diluted in Washing Buffer, for 30 min. at RT. Rinse 2X, 10 min. each at RT with Washing Buffer.
10. Rinse once in TBS.
11. Add Enzyme Substrate and incubate at RT for 5-10 min. The antigen/antibody complex formed is characterized by a red insoluble precipitate. The membrane may have a slight reddish background.
12. Wash in several changes of distilled water.
13. Dry the membrane between sheets of filter paper under cold air stream.
14. Store the peroxidase labeled membrane in the dark in a plastic sleeve.

Notes

1. Membranes processed as described above should be used shortly after preparation. If stored at 4°C, dried membranes may be used within a few weeks, after completion of blocking step (step B6).
2. If broad bands appear on the strips, inspect the opposite side of strip for sharper bands.

References

1. Hulen, D., et al., Cell Motil. Cyto., **18**, 113 (1991).
2. Cohen, P., and Klee, C.B., eds., Calmodulin: Molecular Aspects of Cellular Regulation, Vol. 5, Elsevier, New York (1988).
3. Seto-Ohshima, A., et al., Acta. Histochem. Cytochem., **18**, 275 (1985).
4. Van Eldik, L., and Lukas, T., In: Methods in Enzymology, Means, A., and Conn, P., (eds.), Vol. 139, pp 393, Academic Press, New York (1987).