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

Product No. **C 7055**

Product Description

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 (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

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 kDa 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).

Monoclonal anti-Calmodulin is a homogenous population of antibody molecules that may be used for the localization of calmodulin and study of interactions with biologically active compounds using various immunochemical assays such as ELISA, immunoblot, dot blot and immunocytochemistry.

Calmodulin is a highly conserved 17 kDa calcium-binding protein found in all eukaryotic cells.[2] It is a multifunc- tional, 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 that 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]

Reagent

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 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 hazardous and safe handling practices.

Product Profile

The minimum antibody titer of 1:100 is determined by immunoblotting using a bovine brain calmodulin preparation.

Notes:
1. For sensitive detection of calmodulin bound to membranes, follow the following procedure for immunoblotting of calmodulin.
2. In order to obtain best results in different techniques and preparations, it is recommended that each individual user determine their optimal working dilutions 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.

Procedure for Immunoblotting of Calmodulin[1]

A. Materials

- Membrane: Immobilon PVDF transfer membrane (Product No. P 2563)
- KP Buffer: 25 mM KH_2PO_4/K_2HPO_4 buffer, pH 7.0.
- Absolute methanol

- Transfer buffer: 25 mM Tris + 192 mM glycine, pH 8.3 + 20% methanol
- Fixation Buffer: 0.2% (v/v) glutaraldehyde, freshly prepared in KP Buffer.
- 1 M Lysine in 0.1 M $NaHCO_3$
- PBS (Phosphate Buffered Saline): 10 mM phosphate, 150 mM NaCl, pH 7.5.
- Washing buffer(PBS-T): PBS + 0.05% (v/v) TWEEN® 20
- Blocking Buffer: 5% (w/v) BSA in PBS (use heat-inactivated globin-free BSA, e.g., Product No. A 7638).
- PBS-BSA: 1% BSA in PBS.
- Primary Antibody: Monoclonal Mouse Anti-
- Calmodulin.
- Secondary Antibody: Mouse ExtrAvidin® Peroxidase Staining Kit (Product Code EXTRA-2, consisting of biotinylated antibody and ExtrAvidin-Peroxidase).
- Enzyme Substrate

  Prepare the following stocks and store at 4 °C:
  Solution A: AEC (3-Amino-9-Ethylcarbazole, Product No.A 6926), 20 mg in 2.5 ml DMF (Dimethyl-formamide).
  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.

Notes:

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

B. Procedure

1. Soak SDS polyacrylamide gel for 15 min. in Transfer 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 Transfer Buffer.
3. Conduct semi-dry transfer in Transfer Buffer for 90 min.
4. Incubate the membrane for 60 min. at room temperature (RT) in Fixation Buffer.
5. Wash 3x for 5 min. each in KP buffer.
6. Incubate in 1M Lysine for 60 min.
7. Repeat step 5.
8. Incubate in Blocking Buffer overnight at RT.
9. Rinse once, for 10 min. at RT in washing buffer.
10. Incubate with Primary Antibody, diluted in PBS-BSA for 2 hr.
11. Rinse 3x, 5 min. each at RT using Washing Buffer.
12. Incubate with Biotinylated Secondary Antibody, diluted in Washing Buffer for 60 min.
13. Rinse as in step 11.
14. Incubate with ExtrAvidin-Peroxidase diluted in Washing Buffer, for 60 min. at RT.
15. Rinse as in step 11.
16. 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.
17. Wash in several changes of distilled water.
18. Dry the membrane between sheets of filter paper under cold air stream.
19. 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).
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4. Van Eldik, L.J., and Lukas, T.J., in: Methods in Enzymology, Means, A.R., and P.M. Conn (eds.), **139**, p. 393, Academic Press, New York (1987).

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