

Biomedical Technologies Inc.

bti

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DATA SHEET

CALMODULIN, BOVINE TESTES

Catalog No: BT-372

Lot No: 42994

Quantity: 1mg (lyophilized powder)

Preparation: BTI Calmodulin has been isolated from Bovine Testes and is >99.9% Pure (via Silver Stained, Two Dimensional Polyacrylamide gel electrophoresis).

Biological Assay: 48,000units/mg

Definition: 1 unit of Calmodulin is the amount which will give 50% activation of 0.14 units of bovine retina adenylate cyclase where 1 unit of adenylate cyclase is equivalent to the production of 1 nano-mole of Cyclic AMP per minute per milligram protein at 37°C.

Reconstitution Procedure: Dissolve in pH above seven buffer (ie. tris, borate, etc). Stability can be enhanced by adding Calcium Chloride (100uM).

Storage & Stability: Lyophilized: minimum 6 months at 4°C. Solutions containing Calmodulin should be stored Frozen at -20°C and are stable for 2 months.

Calmodulin Antisera: BTI also offers an affinity purified Sheep anti-Calmodulin IgG (Immunohistochemical Grade), Catalog No: BT-570 made with Calmodulin, Bovine Testes.

References:

- 1 J.R. Dedman, J.D. Potter, A.R. Means, JBC, 252, 2437-2440 (1977).
- 2 R. Gopalkrishna, W.B. Anderson, BBRC, 104, 830-836, (1982).
- 3 M.E. Gnegy, et al, J. of Neuroscience, 4, 2712-2717, (1984).

FOR RESEARCH USE ONLY

Immunohistochemical Localization Procedure

1. Grow cells on glass coverslips (11x22mm).
2. Rinse coverslips in Dulbecco's PBS.
3. Fix cells in 3% Formalin in salt/Pi for 30 minutes at room temperature.
4. Rinse coverslips in salt/Pi and dehydrate cells in absolute acetone at - 20°C for 10 minutes (or absolute acetone (dry ice temp) for 1-3 minutes).
5. Rinse in salt/Pi, 2-3 changes, 5 to 10 minutes per wash with gentle agitation.
6. Invert coverslip over a drop 10-20ul of Calmodulin IgG (approximately 100ug/ml). Incubate for 1 hour at 37°C.
7. Repeat Step 5.
8. Incubate cells with appropriate labelled second antibody (approximately 1:30 dilution) in salt/Pi at 37°C for 45 minutes.
9. Repeat Step 5.
0. Rinse coverslips briefly in distilled water and mount on glass microscope slides in a drop of salt/Pi: glycerol (1:9) pH 8.5.

Immunoblots of Calmodulin

Considerable difficulties can be encountered in immunoblotting Calmodulin. Two publications; one by L.J. Van Eldik, et al¹ (describes the phenomena and offers some potential solutions), and a recent publication by Maxwell T. Hincke² describes in detail *solutions to the problem. It is reported that using potassium buffer in the electro transfer leads to a 6-fold increase in calmodulin retention by nitrocellulose.

The BTI anti-Calmodulin IgG can be used for western immunoblots (1:30-1:150). The exact concentration must be experimentally determined.

*Buffer: 25mM Potassium Phosphate, pH 7.0

- 1.) Van Eldik, L.J. and Wokchok, S.R., "Conditions For Reproducible Detection Of Calmodulin And S100B In Immunoblots", Biomedical And Biophysical Research Communications, 124 No.3, 752-759 (1984).
- 2.) Hincke, Maxwell T., "Conditions for Improved Adsorption of Calmodulin to Nitrocellulose: Detection by Ca⁴⁵ Binding", Electrophoresis, 9, 303-306 (1988).