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

Calmodulin
From bovine testes

Catalog Number **P1431**
Storage Temperature –20 °C

CAS RN 77107-46-1
Synonyms: calcium dependent regulator protein (CDR); phosphodiesterase 3′:5′ cyclic nucleotide activator; CaM

Product Description

Calmodulin is believed to be involved in intracellular Ca^{2+} homeostatis, cell proliferation, smooth muscle contraction, microtubular function, exocytotic secretion of cellular products, and cell motility. Several review articles on calmodulin have been published.[1,2]

Calmoduling contains many acidic amino acids and lacks cysteine, hydroxyproline, and tryptophan. The abundance of acidic carboxyl groups allows for reversibly binding to Ca^{2+}, while the absence of cysteine and hydroxyproline allows for a very flexible tertiary structure for interaction with various calmodulin regulated proteins. Calmodulin also has a high ratio of phenylalanine (8 residues) to tyrosine (2 residues) and has a distinctive UV spectrum with five peaks at 252 nm, 259 nm, 265 nm, 269 nm, and 277 nm, with a shoulder at 282 nm.[3]

X-ray crystallography of calmodulin, in the presence of Ca^{2+}, indicates it exists as a long dumbbell shaped molecule 65 angstroms long. Each globular end contains two Ca^{2+} binding domains. These domains are common among many Ca^{2+} binding proteins and are described as helix-loop-helix “EF-Hand” regions. The Ca^{2+} binding regions are connected by an extended 40 angstroms, 28 amino acid α-helical region.[4-6] Upon Ca^{2+} binding, calmodulin undergoes a conformational change in which the hydrophobic regions become exposed. These hydrophobic regions are believed to be involved with enzyme binding.[7,8] Phosphorylation of calmodulin *in vivo* has been reported when cells are stimulated with insulin[9-11] and *in vitro* by various protein kinases.[12-17]

The four Ca^{2+} binding sites of calmodulin are designated I, II, III, and IV, starting from the site closest to the N-terminus. The order of Ca^{2+} binding to calmodulin is believed to be III, IV, I, and II. Sites III and IV have affinity for Ca^{2+} 10–20 times higher than sites I and II.[18-20]

Molecular mass:
- 16.79 kDa (amino acid sequence)[21]
- 18.7 kDa (sedimentation equilibrium)[22]
- 19 kDa (SDS in presence of EGTA)
- Note: Migration rate in SDS is faster when Ca^{2+} is present and slower when EGTA removes the Ca^{2+}.[23]

Stokes Radius: 20.9 angstroms (calculated)[22]

$E_{276}^{1\%}$ = 1.8 (0.1 M Imidazole-HCl, pH 7.0 with 1 mM EDTA)[24]
Addition of 0.5 mM $CaCl_2$ results in 8% decrease in absorption.[25]

Isoelectric point (pI):[26,27] 3.9–4.3

Calmodulin has been found to activate several enzymes:
- ATPase[28-33]
- Calmodulin Kinase I, II, III [34-38]
- Phosphorylase Kinase[39-42]
- Cyclic Nucleotide Phosphodiesterase[43-46]
- Adenylate Cyclase[47]
- NADPH Oxidase[48]
- Myosin Light Chain Kinase[49]

This product is purified from bovine testes by a modification of a published procedure.[50] It is supplied as an essentially salt free, white lyophilized powder. The calcium content of a representative lot was found to be <0.05%.

Purity: ≥98% (SDS PAGE)

Specific Activity: >40,000 units/ mg protein

Unit Definition: One unit will stimulate 0.008 activated unit of phosphodiesterase 3′:5′-cyclic nucleotide (Catalog Number P0520) in a 3 ml reaction volume at pH 7.5 and 30 °C, to 50% of the maximum activity of the enzyme when saturated with activator, in the presence of 0.01 mM Ca^{2+}.

Calmodulin is assayed spectrophotometrically in a 3.2 ml reaction mixture containing:
66 mM Tris-HCl
54 mM potassium chloride
6.4 mM magnesium sulfate
0.6 mM phospho(enol)pyruvate
0.30 mM adenosine 5′-triphosphate
0.6 mM adenosine 3′:5′-cyclic monophosphate
0.01 mM calcium acetate
11 units pyruvate kinase
16 units L-lactic dehydrogenase
3 units myokinase
0.1 mM β-nicotimamide adenine dinucleotide, reduced
0.0085 unit phosphodiesterase 3′:5′-cyclic nucleotide
1.0 unit of phosphodiesterase 3′:5′-cyclic nucleotide actvitor (calmodulin)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Solutions at 1 mg/ml or higher in water, saline, or 0.1 M Tris-HCl, pH 7.5, (no phosphate or other calcium binding buffers) are stable at 2–8 °C for 1–2 days or frozen for ~1 week.

Storage/Stability
Store the product at –20 °C. Stored properly, as supplied in the powder form, calmodulin should be stable for minimum of two to three years.

References

1. Cheung, W., Science, **207**, 19-27 (1980).
2. Hinrichsen, R., Biochim. Biophys. Acta, **1155**, 277-293 (1993).
3. Cheung, W.Y., Science, **207**, 19, (1980).
4. Babu, S. *et al*., Nature, **315**, 37-40 (1985).
5. Babu, S. *et al*., J. Mol. Biol., **204**, 191-204 (1988).
6. Krestinger, R., in Calcium Transport in Contraction and Secretion, Carafoli, E. et al., eds., pp. 46-478 (1975).
7. Newton, D. *et al*., J. Biol. Chem., **259**, 4419-4426 (1984).
8. Newton, D. *et al*., Biochim. Biophys. Acta, **845**, 533-539 (1985).
9. Sacks, D. *et al*., Biochem. J., **286**, 211-216 (1992).
10. Fukami, Y., *et al*., Proc. Natl. Acad. Sci. USA, **83**, 4190-4193 (1986).
11. Colca, J. *et al*., J. Biol. Chem., **262**, 11399-11402 (1987).
12. Graves, C. *et al*., J. Biol. Chem., **261**, 10429-10438 (1986).
13. Sacks, D., and McDonald, J., J. Biol. Chem., **263**, 2377-2383 (1988).
14. Sacks, D. *et al*., Biochem. J., **262**, 803-812 (1989).
15. Meggio, F. *et al*., FEBS Lett., **215**, 241-246 (1987).
16. Sacks, D. *et al*., Biochem. J., **283**, 21-24 (1992).
17. Sacks, D. *et al*., Biochem. Biophys. Res. Comm., **188**, 754-759 (1992).
18. Olwin, B. *et al*., J. Biol. Chem., **259**, 10949-10955 (1984).
19. Haiech, J. *et al*., J. Biol. Chem., **266**, 3427-3431 (1991).
20. Starovasnik, M. *et al*., Protein Science, **1**, 245-253 (1992).
21. Watterson, D.M., J. Biol. Chem., **255**, 962-975 (1980).
22. Ann. Rev. Biochem., **49**, 493 (1980).
23. Klee, C.B. *et al*., PNAS (USA), **76**, 6270 (1979).
24. Watterson, D.M. *et al*., J. Biol. Chem., **251**, 4501-4513 (1976).
25. Klee, C.B., Biochem., **16**, 1017 (1977).
26. Lin, Y.M. *et al*., J. Biol. Chem., **249**, 4943 (1974).

27. Crouch, T.H. *et al*., Biochem., **19**, 3692-3698 (1980).
28. Watterson, D. *et al*., J. Biol. Chem., **251**, 4501-4513 (1976).
29. Blum, J. *et al*., J. Cell Biol., **87**, 386-397 (1980).
30. Carafoli, E. *et al*., Ann. N.Y. Acad. Sci., **402**, 304-328 (1982).
31. Caroni, P., and Carafoli, E., J. Biol. Chem., **256**, 3263-3270 (1981).
32. McConnell, E.J. *et al*., Circ. Res., **86**, 191-197 (2000).
33. Yingst, D.R. *et al*., Arch. Biochem. Biophys., **295**, 49-54 (1992).
34. Nairn, A., and Greengard, P., J. Biol. Chem., **262**, 7273-7281 (1987).
35. Colbran, R., and Soderling, T., Curr. Top. Cell. Regul., **31**, 181-221 (1990).
36. Nairn, A., and Palfrey, H., J. Biol. Chem., **262**, 17299-17303 (1987).
37. Nairn, A. *et al*., Proc. Natl. Acad. Sci. USA, **82**, 7939-7943 (1985).
38. Krasel, C.J. *et al*., Biol.Chem., **276**,1911-1915 (2001).
39. Newsholme, P. *et al*., J. Biol. Chem., **267**, 810-818 (1992).
40. Chan, K., and Graves, D., J. Biol. Chem., **257**, 948-955 (1982).
41. Cohen, P., Mol. Aspects Cell. Regul., **5**, 123-144 (1988).
42. Juminaga S. *et al*., J. Biol. Chem., **269**, 1660-1667 (1994).
43. Beavo, J. *et al*., Mol. Cell. Endocrinol., **28**, 387-410 (1982).
44. Dedman, J. *et al*., J. Biol. Chem., **252**, 8415-8422 (1977).
45. Cheung, W., *Biochem. Biophys. Res. Commun*., **38**, 533-538 (1970).
46. Rossi, P. *et al*., J. Biol. Chem., **263**, 15521-15527 (1988).
47. Bronstrom, C. *et al*., Proc. Nat. Acad. Sci. USA, **72**, 64-68 (1976).
48. Jones, H. *et al*., Biochim. Biophys. Acta, **714**, 152-156 (1982).
49. Stull, J*., Mol. Aspects Cell. Regul.,* **5**, 91-122 (1988).
50. Gopalakrishna, R., and Anderson, W., Biochem. Biophys. Res. Commun., **104**, 830-836 (1982).

RBG,MAM 05/07-1