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ProductInformation

PHOSPHODIESTERASE 3':5'-CYCLIC NUCLEOTIDE ACTIVATOR
from Bovine Brain

Product Number **P2277**

Cas #: 73298-54-1
Synonyms: calmodulin, calcium dependent regulator protein (CDR), and troponin C - Like peptide.

Product Description
Appearance: White powder.
Molecular weight:
- 16,790 by amino acid sequence[1]
- 18,700 by sedimentation equilibrium[2]
- 19,000 by SDS gel (in presence of EGTA)

NOTE: Migration rate in SDS is faster when Ca+2 is present and slower when EGTA removes the Ca+2.)[3]
Stokes Radius: 20.9 angstroms as calculated from above values[2]
$E^{1\%}$(278nm) = 1.8 Solvent:0.1M Imidazole-HCl - 0.001M EDTA, pH 7.0[4]
If calcium is removed, E1% increases to 2.0[5]
Isoelectric point: The literature reports various pI values ranging from 3.9-4.3[6,7]
Purity: Greater than 98% by SDS PAGE using brilliant blue stain. Supplied as an essentially salt-free powder.
Activity:>40,000 units per mg protein (Lowry)
Structure: Calmodulin has a highly conserved structure and is ubiquitous to eukaryotes.[8,9,10] A Posttranslational modification of most species is a trimethylated lysine at the 115 position,[10,11] and acetylation of the terminal alanine residue.[11]

Calmodulin contains many acidic amino acids. The protein lacks cysteine, hydroxyproline and tryptophan. The abundance of acidic amino acid carboxylate groups allows reversibly binding to calcium 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 ultraviolet spectrum with five peaks at 253 nm, 259 nm, 365 nm, 269 nm and 277 nm, and a shoulder at 282 nm.[11] Phosphorylation of Calmodulin in vivo has been reported when cells are stimulated with insulin,[12,13,14] and in vitro by various protein kinases.[15,16,17,18,19,20]

Xray crystallography of calmodulin, in the presence of calcium, indicates that it exists as a long dumbbell shaped molecule 65 anstroms long. Each globular end contains two calcium binding domains. These domains are common among many calcium binding proteins, and are described as helix-loop-helix "EF-Hand" regions. The calcium binding regions are connected by an extended 40 angstrom, 28 amino acid alpha helical region.[21,22,23] Upon binding to calcium, calmodulin undergoes a conformational change in which the hydrophobic regions become exposed. These hydrophobic regions are believed to be involved with enzyme binding.[24,25]

The four calcium binding sites of calmodulin are designated as I, II, III and IV, starting from the site closest to the amino terminus. The order of calcium to calmodulin is believed to occur in the order of III, IV, I and II. Sites III and IV have an affinity for calcium 10-20 times higher than sites I and II.[26,27,28]

Composition: Calmodulin P2277 is supplied as a salt-free lyophilized powder. The calcium content of a representative lot was found to be less than 0.05%

Applications:
Calmodulin has been found to activate several enzymes.

Cyclic Nucleotide Phosphodiesterase[29, 30, 31]
Nitric Oxide Synthetase[32, 33]
ATPase[34, 35, 36, 37]
Phosphorylase Kinase[38, 39, 40]
Myosin Light-Chain Kinase [41]
Protein Phosphatase (Calcineurin)[42, 43]
Adenylate Cyclase[44]
NADPH Oxidase[45]
CaM Kinases I, II and III [46, 47, 48, 49]

Calmodulin is believed to be involved in intracellular Ca 2+ homeostasis, cell proliferation, smooth muscle contraction, microtubular function, exocytotic secretion of cellular products, and cell motility. Several review articles on calmodulin have been published.[50,51]

Preparation Instructions
Sigma's method of preparation is based on a modification of the procedure of Gopalakrishna.[52] Solutions at 1 mg/mL or higher in water or saline (no phosphate or other calcium binding buffers)at refrigerator temperature are stable for 1-2 days or frozen at 1 mg/mL or higher for approximately one week.

Storage/Stability
Store at less than 0°C. Stored properly, as supplied in the powder form, calmodulin should be stable for a minimum of two to three years.

Product Profile
One unit will stimulate 0.016 activated unit of phosphodiesterase, 3':5' - cyclic nucleotide, P 0520, 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^{++}.

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