

MRC PPU REAGENTS

Standard Operating Procedure

Preparation of Calmodulin 2 [1 - 149]

Enzyme description:- Calmodulin 2 [1 – 149]

Clone number:- DU 11423

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 43, 633.47 daltons

Average Mass 43, 661.75 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.64

Purity:- >85 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 deg C

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Clone Data Sheet

Calmodulin 2 [1 – 149]

<u>Protein</u>	Calmodulin 2 [1 – 149]
<u>Clone number</u>	DU 11423
<u>Species</u>	Human
<u>Accession number</u>	NM_001743
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE GAVLDIRYGVSR IAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKY LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSMADQLTEEQ IAEFKEAFSLFDKGDGTITTKELGTVMRSLGQNPTEAELQDMINEVD ADGNGTIDFPEFLTMMARKMKD TDSEEEIREAFRVFDKDGNGYISAAE LRHVMTNLGEKLTDEEVDEMI READIDGDGQVNYEEFVQMMTAK
<u>Native sequence</u>	Amino acids M1 – K149 (end) of human Calmodulin 2. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 – 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pGEX6P-3

Nucleotide Sequence Of Insert

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ggatccATGGCTGACCAACTGACTGAAGAGCAGATTGCAGAATTCAAAGAAGCT
TTTTCACTATTTGACAAAGATGGTGATGGAACATAACAACAAAGGAATTGGGA
ACTGTAATGAGATCTCTTGGGCAGAAATCCCACAGAAGCAGAGTTACAGGACATG
ATTAATGAAGTAGATGCTGATGGTAATGGCACAATTGACTTCCCTGAATTTCTG
ACAATGATGGCAAGAAAAATGAAAGACACAGACAGTGAAGAAGAAATTAGAGAA
GCATTCCGTGTGTTTGATAAGGATGGCAATGGCTATATTAGTGCTGCAGAACTT
CGCCATGTGATGACAAACCTTGGAGAGAAGTTAACAGATGAAGAAGTTGATGAA
ATGATCAGGGAAGCAGATATTGATGGTGATGGTCAAGTAAACTATGAAGAGTTT
GTACAAATGATGACAGCAAAGtgagcggccgc
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