

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of Calmodulin 2 [1 – 149]**

**Enzyme description:-** Calmodulin 2 [1 – 149]

**Clone number:-** DU 45677

**Source:-** Recombinant

**Expression system:-** *E. coli*

**Tag:-** N-terminal His(6) tag

**Purification method:-** Ni<sub>2+</sub>-NTA agarose

**Calculated molecular mass:-**

Monoisotopic 19, 619.02 daltons

Average Mass 19, 631.58 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 4.39

**Purity:-** 80 %

**Enzyme storage buffer:-**

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,  
0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine

**Storage temperature:-** -70 °C

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**Clone Data Sheet**

**Calmodulin 2 [1 – 149]**

<b><u>Protein</u></b>	Calmodulin 2 [1 – 149]
<b><u>Clone number</u></b>	DU 45677
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_001743
<b><u>Tags</u></b>	N-terminal His(6)
<b><u>Bacterially expressed protein</u></b>	<b>MGSSHHHHHHSSGENLYFQGHMLGSMADQLTEEQIAEFKEAFSLF DKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTID FPEFLTMMARKMKDSDSEEEIREAFRVFDKDGNGYISAAELRHVM TNLGEKLTDEEVDEMIREADIDGDGQVNYEEFVQMMTAK</b>
<b><u>Native sequence</u></b>	Amino acids M1 – K149 (end) of human. Residue M26 of the fusion protein is equivalent to M1 of the native enzyme. The His6 tag is located at residues 5 – 10.
<b><u>Protease cleavage</u></b>	rTEV ( <u>ENLYFQG</u> ) residues 14 - 20
<b><u>Cloning sites</u></b>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pET15b

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**Nucleotide insert**

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ggatccATGGCTGACCAACTGACTGAAGAGCAGATTGCAGAATTC  
AAAGAAGCTTTTTCACTATTTGACAAAGATGGTGATGGAACTATA  
ACAACAAAGGAATTGGGAAGTGAATGAGATCTCTTGGGCAGAAT  
CCCACAGAAGCAGAGTTACAGGACATGATTAATGAAGTAGATGCT  
GATGGTAATGGCACAATTGACTTCCCTGAATTTCTGACAATGATG  
GCAAGAAAATGAAAGACACAGACAGTGAAGAAGAAATTAGAGAA  
GCATTCCGTGTGTTTGATAAGGATGGCAATGGCTATATTAGTGCT  
GCAGAACTTCGCCATGTGATGACAAACCTTGGAGAGAAGTTAACA  
GATGAAGAAGTTGATGAAATGATCAGGGAAGCAGATATTGATGGT  
GATGGTCAAGTAAACTATGAAGAGTTTGTACAAATGATGACAGCA  
AAGtgagcggccgc
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