

Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CaMK2D [1 – 478]

Enzyme description:- CaMK2D [1 - 478]

Clone number:- DU 33795

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 80, 928.15 daltons

Average Mass 80, 980.12 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.41

Purity:- 85 %

Activation protocol:- Constitutively active

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc, 0.1
mM CaCl₂, 1 μM Calmodulin

Substrate:-

YLRRRLSDSNS Final concentration: 300 μM

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Clone Data Sheet

CaMK2D [1 – 478]

<u>Protein</u>	CaMK2D [1 - 478]
<u>Clone number</u>	DU 33795
<u>Species</u>	Human
<u>Accession number</u>	NM_172115.2
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHYERDEGDKWRNKKFELG LEFPNLPLYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESIMLEGA VLDIERYGSRIAYSKDFETLKVDLFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS MASTTCTRFTDEY QLFEELGKGAFSVVRRCMKIPTGQEYAAKIINTKKLSARDHQKLEREAR ICRLLKHPNIVRLHDSISEEGFHYLVDLVTGGELFEDIVAREYYSEAD ASHCIQQILESVNHCNLNGIVHRDLKPENLLLASKSKGVAVKLADFGLA IEVQGDQQAWFGFAGTPGYLSPEVLRKDYGKPVDMWACGVILYILLVG YPPFWDEDQHRLYQQIKAGAYDFPSPEWDTVTPEAKDLINKMLTINPAK RITASEALKHPWICQRSTVASMMHRQETVDCLKKFNARRKLKGAILTTM LATRNFSAAKSLLKKPDGVKESTESSNTTIEDEDVKARKQEIKVTEQL IEAINNGDFEAYTKICDPGLTAFEPEALGNLVEGMDFHRFYFENALSKS NKPIHTIILNPHVHLVGDDAACIAYIRLTOYMDGSGMPKTMQSEETRVW HRRDGKWQNVFHRSGSPTVPIK
<u>Native sequence</u>	Amino acids M1 – K478 (end) of human CaMK2D. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
	The following amino acid substitution is present: A – V , where A151 of native enzyme is V382 of the fusion protein
<u>Protease cleavage</u>	PreScission (<u>LEVLFOGP</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX 6P-1

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<u>Nucleotide</u> <u>Sequence of insert</u>	ggatccATGGCTTCGACCACAACCTGCACCAGGTTACGGACGGTATC AGCTTTGAGGAGCTGGAAAGGGGGCATTCTCAGTGGTGAGAAGATC TATGAAAATTCTACTGGACAAGAATATGCTGCCAAATTATCAACACC AAAAAGCTTCTGCTAGGGATCATCAGAAACTAGAAAGAGAAGCTAGAA TCTGCCGTCTTGAGCACCCTAATATTGTGCGACTTCATGATAGCAT ATCAGAAAGAGGGCTTCACTACTTGGTGTGATTAGTTACTGGAGGT GAACGTGTTGAAGACATAGTGGCAAGAGAATACTACAGTGAAGCTGATG CCAGTCATTGTATACAGCAGATTCTAGAAAGTGTAAATCATTGTCACCT AAATGGCATAGTCACAGGGACCTGAAGCCTGAGAATTGCTTTAGCT AGCAAATCCAAGGGAGTAGCTGTGAAATTGGCAGACCTTGGCTTAGCCA TAGAAGTTCAAGGGGACCAGCAGGGTGGTTGGTTGCTGGCACACC TGGATATCTTCTCCAGAAGTTTACGTAAGATCCTATGGAAAGCCA GTGGATATGTGGGCATGTGGTGTCAATTCTATATTCTACTTGTGGGGT ATCCACCCCTCTGGGATGAAGACCAACACAGACTCTATCAGCAGATCAA GGCTGGAGCTTATGATTTCCATCACCAGAATGGACACGGTGAECTCCT GAAGCCAAAGACCTCATCAATAAAATGTTACTATCAACCTGCCAACAC GCATCACAGCCTCAGAGGCACTGAAGCACCCATGGATCTGTCAACGTT TACTGTTGCTTCCATGATGCACAGACAGGAGACTGTAGACTGCTGAAG AAATTAAATGCTAGAAGAAAACCTAAAGGGTGCCTTGACAACATATGC TGGCTACAAGGAATTCTCAGCAGCCAAGAGTTGTAAGAAACCAGA TGGAGTAAAGGAGTCAACTGAGAGTTCAAATACAACAATTGAGGATGAA GATGTGAAAGCACGAAAGCAAGAGATTATCAAAGTCACTGAACAACTGA TCGAAGCTATCAACAATGGGACTTTGAAGCCTACACAAAAACTGTGA CCCAGGCCTTACTGCTTGAACCTGAAGCTTGGTAATTAGTGGAA GGGATGGATTTCACCGATTCTACTTGAAGGTTGCTTGTCCAAAAGCA ATAAACCAATCCACACTATTATTCTAAACCTCATGTACATCTGGTAGG GGATGATGCCGCCTGCATAGCATATTAGGCTCACACAGTACATGGAT GGCAGTGGAATGCCAAAGACAATGCAGTCAGAAGAGACTCGTGTGTC ACCGCCGGGATGGAAAGTGGCAGAATGTTCATTTCATCGCTCGGGTC ACCAACAGTACCCATCAAGtaagcggccgc
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