Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active calcium / calmodulin-dependent protein kinase IV [1 - 473]

Enzyme description:- CAMK4 [1 - 473]

Clone number:- DU 35125

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 79, 677.99 daltons
Average Mass 79, 728.80 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.71

Purity:- >80 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM $CaCl_2$, 1 μM Calmodulin, 10 mM MgAc,

Substrate:-
KKLNRTLSVA Final concentration: 300 μM

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Clone Data Sheet

calcium / calmodulin-dependent protein kinase IV [1 - 473]

Protein CAMK4 [1 - 473]

Clone number DU 35125

Species Human

Accession number NM_001744.3

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPGIPGSTRA
AA**MLKVTVPSCSASSCSSVTASAAPGTASLVPDYWIDGSNRDALSDFF**
EVESELGRGATSIVYRCKQKGTQKPYALKVLKKTVDKKIVRTEIGVLL
RLSHPNIIKLKEIFETPTEISLVLELVTGGELFDRIVEKGYYSERDAA
DAVKQILEAVAYLHENGIVHRDLKPENLLYATPAPDAPLKIADFGLSK
IVEHQVLMKTVCGTPGYCAPEILRGCAYGPEVDMWSVGIITYILLCGF
EPFYDERGDQFMFRRILNCEYYFISPWWDEVSLNAKDLVRKLIVLDPK
KRLTTFQALQHPWVTGKAANFVHMDTAQKKLQEFNARRKLKAAVKAVV
ASSRLGSASSSHGSIQESHKASRDPSPIQDGNEDMKAIPEGEKIQGDG
AQAAVKGAQAELMKVQALEKVKGADINAEEAPKMVPKAVEDGIKVADL
ELEEGLAEEKLKTVEEAAAPREGQGSSAVGFEVPQQDVILPEY

Native sequence Amino acids M1 – Y473 (end) of human CAMK4.

Residue M243 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage Prescission site (LEVLFQGP) at residues 221 - 228

Cloning sites *Not*1 sites of pGEX-6P-2

Nucleotide sequence of insert

gcggccgcgATGCTCAAAGTCACGGTGCCCTCCTGCTCCGCCTCGTCC
TGCTCTTCGGTCACCGCCAGTGCGGCCCCGGGGACCGCGAGCCTCGTC
CCGGATTACTGGATCGACGGCTCCAACAGGGATGCGCTGAGCGATTTC
TTCGAGGTGGAGTCGGAGCTGGGACGGGGTGCTACATCCATTGTGTAC
AGATGCAAACAGAAGGGGACCCAGAAGCCTTATGCTCTCAAAGTGTTA
AAGAAAACAGTGGACAAAAAAATCGTAAGAACTGAGATAGGAGTTCTT
CTTCGCCTCTCACATCCAAACATTATAAAACTTAAAGAGATATTTGAA
ACCCCTACAGAAATCAGTCTGGTCCTAGAACTCGTCACAGGAGGAGAA
CTGTTTGATAGGATTGTGGAAAAGGGATATTACAGTGAGCGAGATGCT
GCAGATGCCGTTAAACAAATCCTGGAGGCAGTTGCTTATCTACATGAA
AATGGGATTGTCCATCGTGATCTCAAACCAGAGAATCTTCTTTATGCA
ACTCCAGCCCCAGATGCACCACTCAAAATCGCTGATTTTGGACTCTCT
AAAATTGTGGAACATCAAGTGCTCATGAAGACAGTATGTGGAACCCCA
GGGTACTGCGCACCTGAAATTCTTAGAGGTTGTGCCTATGGACCTGAG
GTGGACATGTGGTCTGTAGGAATAATCACCTACATCTTACTTTGTGGA
TTTGAACCATTCTATGATGAAAGAGGCGATCAGTTCATGTTCAGGAGA
ATTCTGAATTGTGAATATTACTTTATCTCCCCCTGGTGGGATGAAGTA
TCTCTAAATGCCAAGGACTTGGTCAGAAAATTAATTGTTTTGGATCCA
AAGAAACGGCTGACTACATTTCAAGCTCTCCAGCATCCGTGGGTCACA
GGTAAAGCAGCCAATTTTGTACACATGGATACCGCTCAAAAGAAGCTC
CAAGAATTCAATGCCCGGCGTAAGCTTAAGGCAGCGGTGAAGGCTGTG
GTGGCCTCTTCCCGCCTGGGAAGTGCCAGCAGCAGCCATGGCAGCATC
CAGGAGAGCCACAAGGCTAGCCGAGACCCTTCTCCAATCCAAGATGGC
AACGAGGACATGAAAGCTATTCCAGAAGGAGAGAAAATTCAAGGCGAT
GGGGCCCAAGCCGCAGTTAAGGGGGCACAGGCTGAGCTGATGAAGGTG
CAAGCCTTAGAGAAAGTTAAAGGTGCAGATATAAATGCTGAAGAGGCC
CCCAAAATGGTGCCCAAGGCAGTGGAGGATGGGATAAAGGTGGCTGAC
CTGGAACTAGAGGAGGGCCTAGCAGAGGAGAAGCTGAAGACTGTGGAG
GAGGCAGCAGCTCCCAGAGAAGGGCAAGGAAGCTCTGCTGTGGGTTTT
GAAGTTCCACAGCAAGATGTGATCCTGCCAGAGTACtaagcggccgc