Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PRKG1 isoform 2 [1 - 686]

Enzyme description:- PRKG1 isoform 2 [1 – 686]

Clone number:- DU 26285

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His6

Purification method:- Ni^{2+}-NTA agarose

Calculated molecular mass:-
Monoisotopic 81, 124.05 daltons
Average Mass 81, 175.47 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.37

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % 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

Substrate:-
KEAKEKRQEQIAKRRRLSSLRASTSKSGGSQK

Final concentration: 300 µM

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Clone Data Sheet

PRKG1 isoform 2 [1 - 686]

Protein	PRKG1 isoform 2 [1 - 686]
Clone number	DU 26285
Species	Human
Accession number	NM_006258.3
Tags	N-terminal His6
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**MGTLRDLQYALQEKIEELRQ RDALIDELELELDQKDELIQKLQNELDKYRSVIRPATQQAQKQSASTL QGEPRTKRQAISAEPTAFDIQDLSHVTLPFYPKSPQSKDLIKEAILDN DFMKNLELSQIQEIVDCMYPVEYGKDSCIIKEGDVGSLVYVMEDGKVE VTKEGVKLCTMGPGKVFGELAILYNCTRTATVKTLVNVKLWAIDRQCF QTIMMRTGLIKHTEYMEFLKSVPTFQSLPEEILSKLADVLEETHYENG EYIIRQGARGDTFFIISKGTVNVTREDSPSEDPVFLRTLGKGDWFGEK ALQGEDVRTANVIAAEAVTCLVIDRDSFKHLIGGLDDVSNKAYEDAEA KAKYEAEAAFFANLKLSDFNIIDTLGVGGFGRVELVQLKSEESKTFAM KILKKRHIVDTRQQEHIRSEKQIMQGAHSDFIVRLYRTFKDSKYLYML MEACLGGELWTILRDRGSFEDSTTRFYTACVVEAFAYLHSKGIIYRDL KPENLILDHRGYAKLVDFGFAKKIGFGKKTWTFCGTPEYVAPEIILNK GHDISADYWSLGILMYELLTGSPPFSGPDPMKTYNIILRGIDMIEFPK KIAKNAANLIKKLCRDNPSERLGNLKNGVKDIQKHKWFEGFNWEGLRK GTLTPPIIPSVASPTDTSNFDSFPEDNDEPPPDDNSGWDIDF**
Native sequence	Amino acids M1 – F686 (end) of human PRKG1 isoform 2. Residue M29 of the fusion protein is equivalent to M1 of the native enzyme. The His6 tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 – 24
Cloning sites	*Bam*H1 and *Not*1 sites of pFastBac HTb

Nucleotide sequence of insert

ggatccATGGGCACCTTGCGGGATTTACAGTACGCGCTCCAGGAGAAG
ATCGAGGAGCTGAGGCAGCGGGATGCTCTCATCGACGAGCTGGAGCTG
GAGTTGGATCAGAAGGACGAACTGATCCAGAAGCTGCAGAACGAGCTG
GACAAGTACCGCTCGGTGATCCGACCAGCCACCCAGCAGGCGCAGAAG
CAGAGCGCGAGCACCTTGCAGGGCGAGCCGCGCACCAAGCGGCAGGCG
ATCTCCGCCGAGCCCACCGCCTTCGACATCCAGGATCTCAGCCATGTG
ACCCTGCCCTTCTACCCCAAGAGCCCACAGTCCAAGGATCTTATAAAG
GAAGCTATCCTTGACAATGACTTTATGAAGAACTTGGAGCTGTCGCAG
ATCCAGGAGATTGTGGATTGTATGTACCCGGTGGAGTATGGCAAGGAC
AGTTGCATCATCAAAGAAGGAGACGTGGGGTCACTGGTGTATGTCATG
GAAGATGGTAAGGTTGAAGTTACAAAAGAAGGTGTGAAGTTGTGTACC
ATGGGTCCAGGAAAAGTGTTTGGGGAATTGGCTATTCTTTACAACTGT
ACCCGGACAGCGACCGTCAAGACTCTTGTAAATGTAAAACTCTGGGCC
ATTGATCGACAATGTTTTCAAACAATAATGATGAGGACAGGACTCATC
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AGCCTTCCTGAAGAGATCCTCAGCAAGCTTGCTGATGTCCTTGAAGAG
ACCCACTATGAAAATGGAGAATATATTATCAGGCAAGGTGCAAGAGGG
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GAGATCATCCTGAACAAAGGCCATGACATTTCAGCCGACTACTGGTCA
CTGGGAATCCTAATGTATGAACTCCTGACTGGCAGCCCACCTTTCTCA
GGCCCAGATCCTATGAAAACCTATAACATCATATTGAGGGGGATTGAC
ATGATAGAATTTCCAAAGAAGATTGCCAAAAATGCTGCTAATTTAATT
AAAAAACTATGCAGGGACAATCCATCAGAAAGATTAGGGAATTTGAAA
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AACGATGAACCACCACCTGATGACAACTCAGGATGGGATATAGACTTC
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