MRC PPU REAGENTS

Standard Operating Procedure

Preparation of active PRKG2 [1 - 762]

Enzyme description:- PRKG2 [1 – 762]

Clone number:- DU 26294

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His6

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

Calculated molecular mass:-
Monoisotopic 90, 746.61 daltons
Average Mass 90, 803.79 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.43

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:-
KKRKSSLRRWSPLTPRQMSFDC Final concentration: 300 μM

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Clone Data Sheet

PRKG2 [1 - 762]

Protein	PRKG2 [1 - 762]
Clone number	DU 26294
Species	Human
Accession number	Q13237.1
Tags	N-terminal His6
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGA**MGSMGNGSVKPKHSKHPDGHSGN LTTDALRNKVTELERELRRKDAEIQEREYHLKELREQLSKQTVAIAEL TEELQNKCIQLNKLQDVVHMQGGSPLQASPDKVPLEVHRKTSGLVSLH SRRGAKAGVSAEPTTRTYDLNKPPEFSFEKARVRKDSSEKKLITDALN KNQFLKRLDPQQIKDMVECMYGRNYQQGSYIIKQGEPGNHIFVLAEGR LEVFQGEKLLSSIPMWTTFGELAILYNCTRTASVKAITNVKTWALDRE VFQNIMRRTAQARDEQYRNFLRSVSLLKNLPEDKLTKIIDCLEVEYYD KGDYIIREGEEGSTFFILAKGKVKVTQSTEGHDQPQLIKTLQKGEYFG EKALISDDVRSANIIAEENDVACLVIDRETFNQTVGTFEELQKYLEGY VANLNRDDEKRHAKRSMSNWKLSKALSLEMIQLKEKVARFSSSSPFQN LEIIATLGVGGFGRVELVKVKNENVAFAMKCIRKKHIVDTKQQEHVYS EKRILEELCSPFIVKLYRTFKDNKYVYMLLEACLGGELWSILRDRGSF DEPTSKFCVACVTEAFDYLHRLGIIYRDLKPENLILDAEGYLKLVDFG FAKKIGSGQKTWTFCGTPEYVAPEVILNKGHDFSVDFWSLGILVYELL TGNPPFSGVDQMMTYNLILKGIEKMDFPRKITRRPEDLIRRLCRQNPT ERLGNLKNGINDIKKHRWLNGFNWEGLKARSLPSPLQRELKGPIDHSY FDKYPPEKGMPPDELSGWDKDF**
Native sequence	Amino acids M1 – F762 (end) of human PRKG2. 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	*Bgl*II and *Not*1 into *Bam*H1 and *Not*1 sites of pFastBac HTb

Complete Nucleotide Sequence

ATGGGAAATGGTTCAGTGAAACCTAAACATTCTAAGCACCCAGATGGA
CACTCTGGGAACCTCACCACTGATGCTCTGCGGAACAAGGTGACAGAG
CTGGAGAGAGAGTTGAGGAGGAAGGATGCTGAGATCCAGGAGCGGGAG
TACCATTTGAAGGAGCTGCGGGAGCAGCTGTCGAAGCAGACTGTGGCC
ATTGCTGAACTCACAGAGGAGCTCCAGAACAAGTGCATCCAGCTGAAC
AAGCTGCAGGATGTGGTGCATATGCAGGGAGGAAGCCCGCTTCAGGCC
TCTCCAGATAAAGTGCCTCTTGAGGTCCACCGGAAGACCTCTGGATTG
GTCTCTCTCCATAGCAGGAGGGGAGCAAAGGCTGGCGTGTCTGCTGAG
CCAACAACCCGGACCTATGACCTGAACAAACCCCCTGAATTTTCCTTT
GAGAAAGCAAGAGTCAGAAAAGACTCCAGTGAGAAGAAGCTCATTACA
GATGCCCTTAATAAAAATCAGTTTCTGAAAAGACTGGATCCTCAGCAG
ATCAAAGACATGGTGGAATGCATGTATGGGAGAAACTATCAGCAAGGG
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GATCACAGCTACTTTGACAAATATCCTCCTGAAAAGGGAATGCCTCCA
GATGAGCTATCAGGCTGGGATAAAGACTTCtgagcggccgc

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