Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active PRK2 [501 - 984]

Enzyme description:- PRK2 [501 - 984]

Clone number:- DU 1658

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6) and FLAG

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

Expression level:- 1-3 mg/L

Calculated molecular mass:- 59, 739 daltons

Purity:- > 90%

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, 0.2 mM PMSF,1 mM Benzamidine.

Storage temperature:- -70 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1% 2−mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-
Long S6 peptide (KEAKEKRQEQIAKRRRLSSLRASTSKSGGSQK)
Final concentration: 30 µM

Specific activity range:- 100 - 200 U/mg

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CLONE DATA SHEET

PRK2 [501 – 984]

Protein	PRK2 [501 – 984]
Clone number	DU 1658
Species	Human
Accession number	S75548
Tags	N-terminal His(6) + FLAG (DYKDDDDK)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGSATMDYKDDDDK**GKTFLRAPQM NINIATWGRLVRRAIPTVNHSGTFSPQAPVPTTVPVVDVRIPQLAPPAS DSTVTKLDFDLEPEPPPAPPRASSLGEIDESSELRVLDIPGQDSETVFD IQNDRNSILPKSQSEYKPDTPQSGLEYSGIQELEDRRSQQRFQFNLQDF RCCAVLGRGHFGKVLLAEYKNTNEMFAIKALKKGDIVARDEVDSLMCEK RIFETVNSVRHPFLVNLFACFQTKEHVCFVMEYAAGGDLMMHIHTDVFS EPRAVFYAACVVLGLQYLHEHKIVYRDLKLDNLLLDTEGFVKIADFGLC KEGMGYGDRTSTFCGTPEFLAPEVLTETSYTRAVDWWGLGVLIYEMLVG ESPFPGDDEEEVFDSIVNDEVRYPRFLSTEAISIMRRLLRRNPERRLGA SEKDAEDVKKHPFFRLIDWSALMDKKVKPPFIPTIRGREDVSNFDDEFT SEAPILTPPREPRILSEEEQEMFRDFDYIADWC**
Native sequence	Amino acids G501 – C984 (end) of human PRK2. Residue G40 of the fusion protein is equilivalent to G501 of the native enzyme. The His(6) tag at located at residues 5 - 10 and the FLAG tag at residues 32 – 39.
Protease cleavage	rTEV site (ENLYFQG) residues 18 – 24
Cloning sites	*Bam*H1 and *Eco*R1 site of pFastBAC HTb

Nucleotide sequence of insert

GGATCCGCCACCATGGACTACAAGGACGACGATGACAAGGGC
AAAACATTTCTCAGAGCTCCTCAAATGAATATTAATATTGCC
ACTTGGGGAAGGCTAGTAAGAAGAGCTATTCCTACAGTAAAT
CATTCTGGCACCTTCAGCCCTCAAGCTCCTGTGCCTACTACA
GTGCCAGTGGTTGATGTACGCATCCCTCAACTAGCACCTCCA
GCTAGTGATTCTACAGTAACCAAATTGGACTTTGATCTTGAG
CCTGAACCTCCTCCAGCCCCACCACGAGCTTCTTCTCTTGGA
GAAATAGATGAATCTTCTGAATTAAGAGTTTTGGATATACCA
GGACAGGATTCAGAGACTGTTTTTGATATTCAGAATGACAGA
AATAGTATACTTCCAAAATCTCAATCTGAATACAAGCCTGAT
ACTCCTCAGTCAGGCCTAGAATATAGTGGTATTCAAGAACTT
GAGGACAGAAGATCTCAGCAAAGGTTTCAGTTTAATCTACAA
GATTTCAGGTGTTGTGCTGTCTTGGGAAGAGGACATTTTGGA
AAGGTGCTTTTAGCTGAATATAAAAACACAAATGAGATGTTT
GCTATAAAAGCCTTAAAGAAAGGAGATATTGTGGCTCGAGAT
GAAGTAGACAGCCTGATGTGTGAAAAAAGAATTTTTGAAACT
GTGAATAGTGTAAGGCATCCCTTTTTGGTGAACCTTTTTGCA
TGTTTCCAAACCAAAGAGCATGTTTGCTTTGTAATGGAATAT
GCTGCCGGTGGGGACCTAATGATGCACATTCATACTGATGTC
TTTTCTGAACCAAGAGCTGTATTTTATGCTGCTTGTGTAGTT
CTTGGGTTGCAGTATTTACATGAACACAAAATTGTTTATAGA
GATTTGAAATTGGATAACTTATTGCTAGATACAGAGGGCTTT
GTGAAAATTGCTGATTTTGGTCTTTGCAAAGAAGGAATGGGA
TATGGAGATAGAACAAGCACATTTTGTGGCACTCCTGAATTT
CTTGCCCCAGAAGTATTAACAGAAACTTCTTATACAAGGGCT
GTAGATTGGTGGGGCCTTGGCGTGCTTATATATGAAATGCTT
GTTGGTGAGTCTCCCTTTCCTGGTGATGATGAAGAGGAAGTT
TTTGACAGTATTGTAAATGATGAAGTAAGGTATCCAAGGTTC
TTATCTACAGAAGCCATTTCTATAATGAGAAGGCTGTTAAGA
AGAAATCCTGAACGGCGCCTTGGGGCTAGCGAGAAAGATGCA
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AGCGCTCTGATGGACAAAAAAGTAAAGCCACCATTTATACCT
ACCATAAGAGGACGAGAAGATGTTAGTAATTTTGATGATGAA
TTTACCTCAGAAGCACCTATTCTGACTCCACCTCGAGAACCA
AGGATACTTTCGGAAGAGGAGCAGGAAATGTTCAGAGATTTT
GACTACATTGCTGATTGGTGTtaagaattc