Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active DAPK1 [1 – 363]

Enzyme description:- DAPK1 [1 – 363]

Clone number:- DU 31113

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:-
Monoisotopic 68, 784.45 daltons
Average Mass 68, 828.22 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.96

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, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- –70 °C

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

Substrate:-
KKLNRTLSFAEPG Final concentration: 300 μM

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Clone Data Sheet

DAPK1 [1 – 363]

Protein	DAPK1 [1 - 363]
Clone number	DU 31113
Species	Human
Accession number	NM_004938.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLF QGPLGSPEF**MTVFRQENVDDYYDTGEELGSGQFAVVKKCREKSTG** **LQYAAKFIKKRRTKSSRRGVSREDIEREVSILKEIQHPNVITLHE** **VYENKTDVILILELVAGGELFDFLAEKESLTEEEATEFLKQILNG** **VYYLHSLQIAHFDLKPENIMLLDRNVPKPRIKIIDFGLAHKIDFG** **NEFKNIFGTPEFVAPEIVNYEPLGLEADMWSIGVITYILLSGASP** **FLGDTKQETLANVSAVNYEFEDEYFSNTSALAKDFIRRLLVKDPK** **KRMTIQDSLQHPWIKPKDTQQALSRKASAVNMEKFKKFAARKKWK** **QSVRLISLCQRLSRSFLSRSNMSVARSDDTLDEEDSFVMKAIIHA** **INDDNVPGLQHL**
Native sequence	Amino acids M1 – L363 of human DAPK1 [Full length protein ends at residue R1430] Residue M235 of the fusion protein is equilivalent to M1 of the native enzyme. The GST tag is located at residues 1 - 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Eco*RI and *Not*I sites of pGEX-6P-1

Nucleotide sequence of insert

gaattcATGACCGTGTTCAGGCAGGAAAACGTGGATGATTACTAC
GACACCGGCGAGGAACTTGGCAGTGGACAGTTTGCGGTTGTGAAG
AAATGCCGTGAGAAAAGCACCGGCCTCCAGTATGCCGCCAAATTC
ATCAAGAAAAGGAGGACTAAGTCCAGCCGGCGGGGTGTGAGCCGC
GAGGACATCGAGCGGGAGGTCAGCATCCTGAAGGAGATCCAGCAC
CCCAATGTCATCACCCTGCACGAGGTCTATGAGAACAAGACGGAC
GTCATCCTGATCTTGGAACTCGTTGCAGGTGGCGAGCTGTTTGAC
TTCTTAGCTGAAAAGGAATCTTTAACTGAAGAGGAAGCAACTGAA
TTTCTCAAACAAATTCTTAATGGTGTTTACTACCTGCACTCCCTT
CAAATCGCCCACTTTGATCTTAAGCCTGAGAACATAATGCTTTTG
GATAGAAATGTCCCCAAACCTCGGATCAAGATCATTGACTTTGGG
TTGGCCCATAAAATTGACTTTGGAAATGAATTTAAAAACATATTT
GGGACTCCAGAGTTTGTCGCTCCTGAGATAGTCAACTATGAACCT
CTTGGTCTTGAGGCAGATATGTGGAGTATCGGGGTAATAACCTAT
ATCCTCCTAAGTGGGGCCTCCCCATTTCTTGGAGACACTAAGCAA
GAAACGTTAGCAAATGTATCCGCTGTCAACTACGAATTTGAGGAT
GAATACTTCAGTAATACCAGTGCCCTAGCCAAAGATTTCATAAGA
AGACTTCTGGTCAAGGATCCAAAGAAGAGAATGACAATTCAAGAT
AGTTTGCAGCATCCCTGGATCAAGCCTAAAGATACACAACAGGCA
CTTAGTAGAAAAGCATCAGCAGTAAACATGGAGAAATTCAAGAAG
TTTGCAGCCCGGAAAAAATGGAAACAATCCGTTCGCTTGATATCA
CTGTGCCAAAGATTATCCAGGTCATTCCTGTCCAGAAGTAACATG
AGTGTTGCCAGAAGCGATGATACTCTGGATGAGGAAGACTCCTTT
GTGATGAAAGCCATCATCCATGCCATCAACGATGACAATGTCCCA
GGCCTGCAGCACCTTtgagcggccgc