Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active DAPK2 [1 – 370]

Enzyme description:- DAPK2 [1 - 370]

Clone number:- DU 31115

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 69, 677.97 daltons
Average Mass 69, 722.26 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.16

Purity:- >80 %

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, 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:-
KKLNRTLSFAEPG Final concentration: 300 μM

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Clone Data Sheet

DAPK2 [1 – 370]

Protein	DAPK2 [1 - 370]
Clone number	DU 31115
Species	Human
Accession number	NM_014326.3
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MFQASMRSPNMEPF KQQKVEDFYDIGEELGSGQFAIVKKCREKSTGLEYAAKFIKKRQSRASR RGVSREEIEREVSILRQVLHHNVITLHDVYENRTDVVLILELVSGGELF DFLAQKESLSEEEATSFIKQILDGVNYLHTKKIAHFDLKPENIMLLDKN IPIPHIKLIDFGLAHEIEDGVEFKNIFGTPEFVAPEIVNYEPLGLEADM WSIGVITYILLSGASPFLGDTKQETLANITAVSYDFDEEFFSQTSELAK DFIRKLLVKETRKRLTIQEALRHPWITPVDNQQAMVRRESVVNLENFRK QYVRRRWKLSFSIVSLCNHLTRSLMKKVHLRPDEDLRNCESDTEEDIAR RKALHPRRRSSTS**
Native sequence	Amino acids M1 – S370 (end) of human DAPK2. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 229
Cloning sites	*Bam*H1 and *Not*1sites of pGEX 6P-1

Nucleotide Sequence of insert

ggatccATGTTCCAGGCCTCAATGAGGAGTCCAAACATGGAGCCATTCA
AGCAGCAGAAGGTGGAGGACTTTTATGACATCGGAGAGGAGCTGGGGAG
TGGCCAGTTTGCCATCGTGAAGAAGTGCCGGGAGAAGAGCACGGGGCTT
GAGTATGCAGCCAAGTTCATCAAGAAGCGGCAGAGCCGGGCGAGCCGGC
GCGGTGTGAGCCGGGAGGAGATCGAGCGGGAGGTGAGCATCCTGCGGCA
GGTGCTGCACCACAATGTCATCACGCTGCACGACGTCTATGAGAACCGC
ACCGACGTGGTGCTCATCCTTGAGCTAGTGTCTGGAGGAGAGCTCTTCG
ATTTCCTGGCCCAGAAGGAGTCACTGAGTGAGGAGGAGGCCACCAGCTT
CATTAAGCAGATCCTGGATGGGGTGAACTACCTTCACACAAAGAAAATT
GCTCACTTTGATCTCAAGCCAGAAAACATTATGTTGTTAGACAAGAATA
TTCCCATTCCACACATCAAGCTGATTGACTTTGGTCTGGCTCACGAAAT
AGAAGATGGAGTTGAATTTAAGAATATTTTTGGGACGCCGGAATTTGTT
GCTCCAGAAATTGTGAACTACGAGCCCCTGGGTCTGGAGGCTGACATGT
GGAGCATAGGCGTCATCACCTACATCCTCTTAAGTGGAGCATCCCCTTT
CCTGGGAGACACGAAGCAGGAAACACTGGCAAATATCACAGCAGTGAGT
TACGACTTTGATGAGGAATTCTTCAGCCAGACGAGCGAGCTGGCCAAGG
ACTTTATTCGGAAGCTTCTGGTTAAAGAGACCCGGAAACGGCTCACAAT
CCAAGAGGCTCTCAGACACCCCTGGATCACGCCGGTGGACAACCAGCAA
GCCATGGTGCGCAGGGAGTCTGTGGTCAATCTGGAGAACTTCAGGAAGC
AGTATGTCCGCAGGCGGTGGAAGCTTTCCTTCAGCATCGTGTCCCTGTG
CAACCACCTCACCCGCTCGCTGATGAAGAAGGTGCACCTGAGGCCGGAT
GAGGACCTGAGGAACTGTGAGAGTGACACTGAGGAGGACATCGCCAGGA
GGAAAGCCCTCCACCCACGGAGGAGGAGCAGCACCTCCtaagcggccgc