Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active DAPK3 [1 – 404]

Enzyme description:- DAPK3 [1 – 404]

Clone number:- DU 32285

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 79, 310.01 daltons
Average Mass 79, 359.87 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.16

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

DAPK3 [1 – 404]

Protein	DAPK3 [1 - 404]
Clone number	DU 32285
Species	Human
Accession number	NM_001348.1
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLF QGPLGS**MSTFRQEDVEDHYEMGEELGSGQFAIVRKCRQKGTGKEY** **AAKFIKKRRLSSSRRGVSREEIEREVNILREIRHPNIITLHDIFE** **NKTDVVLILELVSGGELFDFLAEKESLTEDEATQFLKQILDGVHY** **LHSKRIAHFDLKPENIMLLDKNVPNPRIKLIDFGIAHKIEAGNEF** **KNIFGTPEFVAPEIVNYEPLGLEADMWSIGVITYILLSGASPFLG** **ETKQETLTNISAVNYDFDEEYFSNTSELAKDFIRRLLVKDPKRRM** **TIAQSLEHSWIKAIRRRNVRGEDSGRKPERRRLKTTRLKEYTIKS** **HSSLPPNNSYADFERFSKVLEEAAAAEEGLRELQRSRRLCHEDVE** **ALAAIYEEKEAWYREESDSLGQDLRRLRQELLKTEALKRQAQEEA** **KGALLGTSGLKRRFSRLENRYEALAKQVASEMRFVQDLVRALEQE** **KLQGVECGLR**
Native sequence	Amino acids M1 – R404 of human DAPK3. Residue M232 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	*Bam*H1 sites of pGEX-6P-1

Nucleotide sequence of insert

ggatccATGTCCACGTTCAGGCAGGAGGACGTGGAGGACCATTAT
GAGATGGGGGAGGAGCTGGGCAGCGGCCAGTTTGCGATCGTGCGG
AAGTGCCGGCAGAAGGGCACGGGCAAGGAGTACGCAGCCAAGTTC
ATCAAGAAGCGCCGCCTGTCATCCAGCCGGCGTGGGGTGAGCCGG
GAGGAGATCGAGCGGGAGGTGAACATCCTGCGGGAGATCCGGCAC
CCCAACATCATCACCCTGCACGACATCTTCGAGAACAAGACGGAC
GTGGTCCTCATCCTGGAGCTGGTCTCTGGCGGGGAGCTCTTTGAC
TTCCTGGCGGAGAAGGAGTCGCTGACGGAGGACGAGGCCACCCAG
TTCCTCAAGCAGATCCTGGACGGCGTTCACTACCTGCACTCTAAG
CGCATCGCACACTTTGACCTGAAGCCGGAAAACATCATGCTGCTG
GACAAGAACGTGCCCAACCCACGAATCAAGCTCATCGACTTCGGC
ATCGCGCACAAGATCGAGGCGGGGAACGAGTTCAAGAACATCTTC
GGCACCCCGGAGTTTGTGGCCCCAGAGATTGTGAACTATGAGCCG
CTGGGCCTGGAGGCGGACATGTGGAGCATCGGTGTCATCACCTAT
ATCCTCCTGAGCGGTGCATCCCCGTTCCTGGGCGAGACCAAGCAG
GAGACGCTCACCAACATCTCAGCCGTGAACTACGACTTCGACGAG
GAGTACTTCAGCAACACCAGCGAGCTGGCCAAGGACTTCATTCGC
CGGCTGCTCGTCAAAGATCCCAAGCGGAGAATGACCATTGCCCAG
AGCCTGGAACATTCCTGGATTAAGGCGATCCGGCGGCGGAACGTG
CGTGGTGAGGACAGCGGCCGCAAGCCCGAGCGGCGGCGCCTGAAG
ACCACGCGTCTGAAGGAGTACACCATCAAGTCGCACTCCAGCTTG
CCGCCCAACAACAGCTACGCCGACTTCGAGCGCTTCTCCAAGGTG
CTGGAGGAGGCGGCGGCCGCCGAGGAGGGCCTGCGCGAGCTGCAG
CGCAGCCGGCGGCTCTGCCACGAGGACGTGGAGGCGCTGGCCGCC
ATCTACGAGGAGAAGGAGGCCTGGTACCGCGAGGAGAGCGACAGC
CTGGGCCAGGACCTGCGGAGGCTACGGCAGGAGCTGCTCAAGACC
GAGGCGCTCAAGCGGCAGGCGCAGGAGGAGGCCAAGGGCGCGCTG
CTGGGGACCAGCGGCCTCAAGCGCCGCTTCAGCCGCCTGGAGAAC
CGCTACGAGGCGCTGGCCAAGCAAGTAGCCTCCGAGATGCGCTTC
GTGCAGGACCTCGTGCGCGCCCTGGAGCAGGAGAAGCTGCAGGGC
GTGGAGTGCGGGCTGCGCtagggatcc