Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active eIF2AK3 [536 – 1116]

Enzyme description:- eIF2AK3 [536 - 1116]

Clone number:- DU 33545

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 93, 477.28 daltons
Average Mass 93, 537.10 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.64

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, 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:-
SRPKtide [RSRSRSRSRSRSRSR] Final concentration: 300 μM

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Clone Data Sheet

eIF2AK3 [536 – 1116]

Protein	eIF2AK3 [536 - 1116]
Clone number	DU 33545
Species	Human
Accession number	NM_004836.5
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPNSRVD**TTFIVRRL** **FHPHPHRQRKESETQCQTENKYDSVSGEANDSSWNDIKNSGYISRYLTD** **FEPIQCLGRGGFGVVFEAKNKVDDCNYAIKRIRLPNRELAREKVMREVK** **ALAKLEHPGIVRYFNAWLEAPPEKWQEKMDEIWLKDESTDWPLSSPSPM** **DAPSVKIRRMDPFSTKEHIEIIAPSPQRSRSFSVGISCDQTSSSESQFS** **PLEFSGMDHEDISESVDAAYNLQDSCLTDCDVEDGTMDGNDEGHSFELC** **PSEASPYVRSRERTSSSIVFEDSGCDNASSKEEPKTNRLHIGNHCANKL** **TAFKPTSSKSSSEATLSISPPRPTTLSLDLTKNTTEKLQPSSPKVYLYI** **QMQLCRKENLKDWMNGRCTIEERERSVCLHIFLQIAEAVEFLHSKGLMH** **RDLKPSNIFFTMDDVVKVGDFGLVTAMDQDEEEQTVLTPMPAYARHTGQ** **VGTKLYMSPEQIHGNSYSHKVDIFSLGLILFELLYPFSTQMERVRTLTD** **VRNLKFPPLFTQKYPCEYVMVQDMLSPSPMERPEAINIIENAVFEDLDF** **PGKTVLRQRSRSLSSSGTKHSRQSNNSHSPLPSN**
Native sequence	Amino acids T536 – N1116 (end) of human eIF2AK3. Residue T238 of the fusion protein is equivalent to T536 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 229
Cloning sites	*Sal*I and *Not*1 sites of pGEX 6P-1

Nucleotide Sequence of insert

gtcgacACAACGTTTATTGTGCGCAGGCTTTTCCATCCTCATCCTCACA
GGCAAAGGAAGGAGTCTGAAACTCAGTGTCAAACTGAAAATAAATATGA
TTCTGTAAGTGGTGAAGCCAATGACAGTAGCTGGAATGACATAAAAAAC
TCTGGATATATATCACGATATCTAACTGATTTTGAGCCAATTCAATGCC
TGGGACGTGGTGGCTTTGGAGTTGTTTTTGAAGCTAAAAACAAAGTAGA
TGACTGCAATTATGCTATCAAGAGGATCCGTCTCCCCAATAGGGAATTG
GCTCGGGAAAAGGTAATGCGAGAAGTTAAAGCCTTAGCCAAGCTTGAAC
ACCCGGGCATTGTTAGATATTTCAATGCCTGGCTCGAAGCACCACCAGA
GAAGTGGCAAGAAAAGATGGATGAAATTTGGCTGAAAGATGAAAGCACA
GACTGGCCACTCAGCTCTCCTAGCCCAATGGATGCACCATCAGTTAAAA
TACGCAGAATGGATCCTTTCTCTACAAAAGAACATATTGAAATCATAGC
TCCTTCACCACAAAGAAGCAGGTCTTTTTCAGTAGGGATTTCCTGTGAC
CAGACAAGTTCATCTGAGAGCCAGTTCTCACCACTGGAATTCTCAGGAA
TGGACCATGAGGACATCAGTGAGTCAGTGGATGCAGCATACAACCTCCA
GGACAGTTGCCTTACAGACTGTGATGTGGAAGATGGGACTATGGATGGC
AATGATGAGGGGCACTCCTTTGAACTTTGTCCTTCTGAAGCTTCTCCTT
ATGTAAGGTCAAGGGAGAGAACCTCCTCTTCAATAGTATTTGAAGATTC
TGGCTGTGATAATGCTTCCAGTAAAGAAGAGCCGAAAACTAATCGATTG
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TGAGCCCAGAGCAGATTCATGGAAACAGCTATTCTCATAAAGTGGACAT
CTTTTCTTTAGGCCTGATTCTATTTGAATTGCTGTATCCATTCAGCACT
CAGATGGAGAGAGTCAGGACCTTAACTGATGTAAGAAATCTCAAATTTC
CACCATTATTTACTCAGAAATATCCTTGTGAGTACGTGATGGTTCAAGA
CATGCTCTCTCCATCCCCCATGGAACGACCTGAAGCTATAAACATCATT
GAAAATGCTGTATTTGAGGACTTGGACTTTCCAGGAAAAACAGTGCTCA
GACAGAGGTCTCGCTCCTTGAGTTCATCGGGAACAAAACATTCAAGACA
GTCCAACAACTCCCATAGCCCTTTGCCAAGCAATtaggcggccgc