Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active NEK3 [1 - 506]

Enzyme description:- NEK3 [1 - 506]

Clone number:- DU 41229

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 84, 474.84 daltons
Average Mass 84, 528.83 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.33

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, 10 mM DTT, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc

Substrate:-
Myelin Basic Protein Final concentration: 0.33 mg/ml

Clone Data Sheet

NEK3 [1 - 506]

Protein	NEK3 [1 - 506]
Clone number	DU 41229
Species	Human
Accession number	NM_002498.2
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNK KFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKE RAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKM FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL VCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD LEVLFQGPLGS**MDDYMVLRMIGEGSFGRALLVQHESSNQMFAMK EIRLPKSFSNTQNSRKEAVLLAKMKHPNIVAFKESFEAEGHLYI VMEYCDGGDLMQKIKQQKGKLFPEDMILNWFTQMCLGVNHIHKK RVLHRDIKSKNIFLTQNGKVKLGDFGSARLLSNPMAFACTYVGT PYYVPPEIWENLPYNNKSDIWSLGCILYELCTLKHPFQANSWKN LILKVCQGCISPLPSHYSYELQFLVKQMFKRNPSHRPSATTLLS RGIVARLVQKCLPPEIIMEYGEEVLEEIKNSKHNTPRKKTNPSR IRIALGNEASTVQEEEQDRKGSHTDLESINENLVESALRRVNRE EKGNKSVHLRKASSPNLHRRQWEKNVPNTALTALENASILTSSL TAEDDRGGSVIKYSKNTTRKQWLKETPDTLLNILKNADLSLAFQ TYTIYRPGSEGFLKGPLSEETEASDSVDGGHDSVILDPERLEPG LDEEDTDFEEEDDNPDWVSELKKRAGWQGLCDR**
Native sequence	Amino acids M1 – R506 (end residue) of human NEK3. 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 - 228
Cloning sites	*Bgl*II and *Not*1sites of pFastBac Dual.

Nucleotide sequence of insert

agatctATGGATGACTACATGGTCCTGAGAATGATTGGGGAGGGC
TCCTTCGGCAGAGCTCTTTTGGTTCAGCATGAAAGCAGTAATCAG
ATGTTTGCCATGAAAGAAATAAGGCTTCCCAAGTCTTTCTCTAAT
ACACAGAATTCTAGGAAGGAGGCTGTTCTTTTAGCCAAAATGAAA
CACCCTAATATTGTTGCCTTCAAAGAATCATTTGAAGCTGAAGGA
CACTTGTATATTGTGATGGAATACTGTGATGGAGGGGATCTAATG
CAAAAGATTAAACAGCAGAAAGGAAAGTTATTTCCTGAAGACATG
ATACTTAATTGGTTTACCCAAATGTGCCTTGGAGTAAATCACATT
CACAAGAAACGTGTGCTACACAGAGATATCAAGTCCAAGAATATC
TTCCTCACTCAGAATGGAAAAGTGAAATTGGGAGACTTTGGATCT
GCCCGTCTTCTCTCCAATCCGATGGCATTTGCTTGTACCTATGTG
GGAACTCCTTATTATGTGCCTCCAGAAATTTGGGAAAACCTGCCT
TATAACAATAAAAGTGACATCTGGTCCTTGGGTTGCATCCTGTAT
GAACTCTGTACCCTTAAGCATCCATTTCAGGCAAATAGTTGGAAA
AATCTTATCCTCAAAGTATGTCAAGGGTGCATCAGTCCACTGCCG
TCTCATTACTCCTATGAACTTCAGTTCCTAGTCAAGCAGATGTTT
AAAAGGAATCCCTCACATCGCCCCTCGGCTACAACGCTTCTCTCT
CGAGGCATCGTAGCTCGGCTTGTCCAGAAGTGCTTACCCCCCGAG
ATCATCATGGAATATGGTGAGGAAGTATTAGAAGAAATAAAAAAT
TCGAAGCATAACACACCAAGAAAAAAAACAAACCCCAGCAGAATC
AGGATAGCTTTGGGAAATGAAGCAAGCACAGTGCAAGAGGAAGAA
CAAGATAGAAAGGGTAGCCATACTGATTTGGAAAGCATTAATGAA
AATTTAGTTGAAAGTGCATTGAGAAGAGTAAACAGAGAAGAAAAA
GGTAATAAGTCAGTCCATCTGAGGAAAGCCAGTTCACCAAATCTT
CATAGACGACAGTGGGAGAAAAATGTACCCAATACAGCTCTTACA
GCTTTGGAAAATGCATCCATACTCACCTCCAGTTTAACAGCAGAG
GACGATAGAGGTGGTTCTGTAATAAAGTACAGCAAAAATACTACT
CGTAAGCAGTGGCTCAAAGAGACCCCTGACACTTTGTTGAACATC
CTTAAGAATGCTGATCTCAGCTTGGCTTTTCAAACATACACAATA
TATAGACCAGGTTCAGAAGGGTTCTTGAAAGGCCCCCTGTCTGAA
GAAACAGAAGCATCGGACAGTGTTGATGGAGGTCACGATTCTGTC
ATTTTGGATCCAGAGCGACTTGAGCCTGGGCTAGATGAGGAGGAC
ACGGACTTTGAGGAGGAAGATGACAACCCCGACTGGGTGTCAGAG
CTGAAGAAGCGAGCTGGATGGCAAGGCCTGTGCGACAGAtaagcg
gccgc