

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of active NEK1 [1 - 1286]**

<b><u>Enzyme description:-</u></b>	NEK1 [1 - 1286]
<b><u>Clone number:-</u></b>	DU 41383
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system
<b><u>Tag:-</u></b>	N-terminal GST
<b><u>Purification method:-</u></b>	GSH Sepharose

#### **Calculated molecular mass:-**

Monoisotopic            84, 297.66 daltons  
Average Mass            84, 351.23 daltons  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-**                            9.11

**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

*Division of Signal Transduction Therapy*

**Clone Data Sheet**

**NEK1 [1 - 1286]**

**Protein** NEK1 [1 - 1286]  
**Clone number** DU 41383  
**Species** Human  
**Accession number** NM\_001199397.1  
**Tags** N-terminal GST

**Baculovirus  
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNK  
KFELGLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKE  
RAEISMLEGAVLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKM  
FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL  
VCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD  
LEVLFOGPLGSMEKYVRLQKIGEGSFGKAILVKSTEDGRQYVIK  
**EINISRMSSKEREESRREVAVLANMKHPNIVQYRESFEENGLY**  
**IVMDYCEGGDLFKRINAQKGVLFQEDQILDWFVQICLALKHVHD**  
**RKILHRDIKSONIFLTKDGTVQLGDFGIARVLNSTVELARTCIG**  
**TPYYLSPEICENKPYNNKSDIWALGCVLYELCTLKHAFEAGSMK**  
**NLVLKIIISGSFPPVSLHYSYDLRSLVSQLFKRNPRDRPSVNSIL**  
**EKGFIAKRIEKFLSPOLIAEEFCLKTFSKFGSQPIPAKRPASGQ**  
**NSISVMPAQKITKPAKYGIPLAYKKYGDKKLHEKKPLQKHQA**  
**HQTPEKRVNTGEERRKISEEAARKRRLEFIEKEKKQKDQIISLM**  
**KAEQMKRQEKERLERINRAREQGWRNVLSAGGSGEVKAPFLGSG**  
**GTIAPSSFSSRGQYEHYHAIFDOMQQORAEDNEAKWKREIYGRG**  
**LPERGILPGVRPGFPYGAAGHHHPDADDIRKTLKRLKAVSKQA**  
**NANRQKQQLAVERAKQVEEFLQRKREAMQNKARAEHMGILQNL**  
**AAMYGGRPSSSRGGKPRNKEEEVYLARLRQIRLQNFNERQQIKA**  
**KLRGEKKEANHSEGQEGSEADMRRKKIESLKAHANARA AVLKE**  
**QLERKRKEAYEREKKVWEHLVAKGVKSSDVSPPLGQHETGGSP**  
**SKQQMRSVISVTSALKEVGVDSSSLTDRETSEEMQKTNNAISSK**  
**REILRRLNENLKAQEDKGNLSDTFEINVHEDAHEHEKESV**  
**SSDRKKWEAGGQLVIPLDELTLDTSFSTTERHTVGEVIKLGPNQ**  
**SPRAWGKSPTDSVLKILGEAELQLOTELLENTTIRSEISPEGE**  
**KYKPLITGEKKVQCISHEINPSAIVDSPVETKSPFSEASPQMS**  
**LKLEGNLEEPDDLETEILQEPSGTNKDES L PCTITD V WISEEKE**  
**TKETQSADRITIQENEVSEDGVSSSTVDQLSDIHIEPGTNSQHS**  
**KCDVDKSVQPEPFFHKVVHSEHLNLVPQVQSVQCSPEESFAFRS**

## *Division of Signal Transduction Therapy*

**HSHLPPKNKNKNSLLIGLSTGLFDANNPKMLRTCSLPDLSKLFRTLMDVPTVGDVRQDNLEIDEIEDENIKEGPSDESDIVFEETDLDLOELQASMEQLLREQPGEEYSEEEESVLKNSDVEPTANGTDVAD EDDNPSSSESALNEEWHSDNSDGEIASECECDSVFNHLEELRLHLEQEMGFEEKFFEVYEKIKAIHEDEDENIEICSKIVQNILGNEHQHLYAKILHLVMADGAYQEDNDE**

- Native sequence**      Amino acids M1 – E1286 (end residue) of human NEK1. 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**      *Bam*H1 and *Not*I sites of pFastBac Dual.

## *Division of Signal Transduction Therapy*

### **Nucleotide sequence of insert**

ggatccATGGAGAAGTATGTTAGACTACAGAAGATTGGAGAAGGTTTCATT  
TGGAAAAGCCATTCTTGTAAATCTACAGAAGATGGCAGACAGTATGTTA  
TCAAGGAAATTAACATCTCAAGAATGTCCAGTAAAGAAAAGAGAAGAATCA  
AGGAGAGAAGTTGCAGTATTGGCAAACATGAAGCATCCAAATATTGTCCA  
GTATAGAGAATCATTGAAGAAAATGGCTCTCTACATAGTAATGGATT  
ACTGTGAGGGAGGGATCTGTTTAAAGCGAATAAATGCTCAGAAAGCGGTT  
TTGTTTCAAGAGGATCAGATTTTGGACTGGTTTGTACAGATATGTTTGGC  
CCTGAAACATGTACATGATAGAAAAATTCCTTCATCGAGACATTAAATCTC  
AGAACATATTTTTAACTAAAGATGGAACAGTACAACCTTGGAGATTTTGG  
ATTGCTAGAGTTCTTAATAGTACTGTAGAGCTGGCTCGAACTTGCATAGG  
GACCCATACTACTTGTCCACTGAAATCTGTGAAAACAAACCTTACAATA  
ATAAAAGTGACATTTGGGCTCTGGGGTGTGTCTTTTATGAGCTGTGTACA  
CTTAAACATGCTTTTGAAGCTGGCAGTATGAAAAACCTGGTACTGAAGAT  
AATATCTGGATCTTTTCCACCTGTGTCTTTGCATTATTCCATGATCTCC  
GCAGTTTGGTGTCTCAGTTATTTAAAAGAAATCCTAGGGATAGACCATCA  
GTCAACTCCATATTGGAGAAAGGTTTATAGCCAAACGCATTGAAAAGTT  
TCTCTCTCCTCAGCTTATTGCAGAAGAATTTTGTCTAAAAACATTTTCGA  
AGTTTGGATCACAGCCTATACCAGCTAAAAGACCAGCTTACAGGACAAAAC  
TCGATTTCTGTTATGCCTGCTCAGAAAATTAACAAAGCCTGCCGCTAAATA  
TGGAAATACCTTTAGCATATAAGAAATATGGAGATAAAAAATTAACACGAAA  
AGAAACCCTGCAAAAACATAAACAGGCCCATCAAACCTCCAGAGAAGAGA  
GTGAATACTGGAGAAGAAAGGAGGAAAATATCTGAGGAAGCAGCAAGAAA  
GAGAAGGCTGGAATTTATTGAAAAAGAAAAGAAAACAAAAGGATCAGATTA  
TTAGTTTAAATGAAGGCTGAACAAATGAAAAGGCAAGAAAAGGAAAGGTTG  
GAAAGAATAAATAGGGCCAGGGAACAAGGATGGAGAAATGTGCTAAGTGC  
TGGTGGAAAGTGGTGAAGTAAAGGCTCCTTTTCTGGGCAGTGGAGGGACTA  
TAGCTCCATCATCTTTTTCTTCTCGAGGACAGTATGAACATTACCATGCC  
ATTTTTGACCAAATGCAGCAACAAGAGCAGAAGATAATGAAGCTAAATG  
GAAAAGAGAAATATATGGTCGAGGCTTCCAGAAAAGGAAATTTCTGCCGTG  
GAGTTCCGTCCAGGATTTCTTTATGGGGCTGCAGGTCATCACCATTTTCTCT  
GATGCTGATGATATTAGAAAACTTTGAAAAGATTGAAGGCGGTGTCTAA  
ACAAGCCAATGCAAACAGGCAAAAAGGGCAGCTAGCTGTAGAAAGAGCTA  
AACAAGTAGAAGAGTTCTTGCAGCGAAAACGGGAAGCTATGCAGAATAAA  
GCTCGAGCCGAAGGACATATGGGAATCCTGCAAAACCTGGCAGCTATGTA  
TGGAGGCAGGCCAGCTCTTCAAGAGGAGGGAAGCCAAGAAACAAAGAGG  
AAGAGGTTTATCTGGCAAGACTGAGGCAATAAGACTACAGAATTTCAAT  
GAGCGCCAACAGATTAAGCCAAACTTCTGTGGTGAAGAAGAAAGCTAA  
TCATTTCTGAAGGACAAGAAGGAAGTGAAGAGGCTGACATGAGGCGCAAAA  
AAATCGAATCACTGAAGGCCCATGCAAATGCACGTGCTGCTGTACTAAAA  
GAACAAC TAGAACGAAAGAGAAAGGAGGCTTATGAGAGAGAAAAAAAGT  
GTGGGAAGAGCATTGGTGGCTAAAGGAGTTAAGAGTTCTGATGTTTCTC  
CACCTTTGGGACAGCATGAAACAGGTGGCTCTCCATCAAAGCAACGATG  
AGATCTGTTATTTCTGTAACCTCAGCTTTGAAAGAAGTTGGCGTGGACAG  
TAGTTTAACTGATACCCGGGAACTT CAGAAGAGATGCAAAAGACCAACA  
ATGCTATTTCAAGTAAGCGAGAAATACTTCTGTAGATTAATGAAAATCTT  
AAAGCTCAAGAAGATGAAAAGGAAAGCAGAATCTCTCTGATACTTTTGA  
GATAAATGTTTCATGAAGATGCCAAAGAGCATGAAAAGAAAAAATCAGTTT  
CATCTGATCGCAAGAAGTGGGAGGCAGGAGGTCAACTTGTGATTTCTCTG  
GATGAGTTAACACTAGATACATCCTTCTCTACAACCTGAAAGACATACAGT  
GGGAGAAGTTATTAATTAGGTCTTAATGGATCTCCAAGAAGAGCCTGGG  
GGAAAAGTCCGACAGATTTCTGTTCTAAAGATACTTGGAGAAGCTGAACTA  
CAACTTCAGACAGAACTATTAGAAAATACAACCTATTAGAAGTGAGATTTT  
TCCCGAAGGGGAAAAGTACAAACCTTAATTACTGGAGAAAAAAAAGTAC  
AATGTATTTACATGAAATAAACCCATCAGCTATTGTTGATTTCTCTGTT  
GAGACAAAAAGTCCCGAGTT CAGTGAGGCATCTCCACAGATGTCAATTGAA  
ACTGGAAGGAAATTTAGAAGAACC TGATGATTTGGAAACAGAAATTTCTAC  
AAGAGCCAAGTGGAAACAAACAAAGATGAGAGCTTGCCATGCATATTACT  
GATGTGTGGATTAGTGAGGAAAAAGAAACAAAGGAAACTCAGTCCGCAGA  
TAGGATCACCATT CAGGAAAATGAAGTTTCTGAAGATGGAGTCTCGAGTA  
CTGTGGACCAACTTAGTGACATTT CATATAGAGCCTGGAACCAATGATTTCT  
CAGCACTCTAAATGTGATGTAGATAAGTCTGTGCAACCGGAACCATTTTT  
CCATAAGGTGGTTCTTCTGAACTTGAACCTTAGTCCCTCAAGTTCAAT  
CAGTTCAGTGTTCACCAGAAGAATCCTTTGCATTTTCGATCTCACTCGCAT

*Division of Signal Transduction Therapy*

TTACCACCAAAAAATAAAAAACAAGAATTCCTTGCTGATTGGACTTTCAAC  
TGGTCTGTTTTGATGCAACAACCCAAAGATGTTAAGGACATGTTCACTTC  
CAGATCTCTCAAAGCTGTTTCAAGAACCCCTTATGGATGTTCCCACCGTAGGA  
GATGTTTCGTCAAGACAATCTTGAAATAGATGAAATTGAAGATGAAAACAT  
TAAAGAAGGACCTTCTGATTCTGAAGACATTGTGTTTGAAGAACTGACA  
CAGATTTACAAGAGCTGCAGGCCTCGATGGAACAGTTACTTAGGGAACAA  
CCTGGTGAAGAATACAGTGAAGAAGAAGAGTCAGTCTTGAAGAACAGTGA  
TGTGGAGCCAAC TGCAAAATGGGACAGATGTGGCAGATGAAGATGACAATC  
CCAGCAGTAAAAGTGCCCTGAACGAAGAATGGCACTCAGATAACAGTGAT  
GGTGAATTGCTAGTGAATGTGAATGCGATAGTGTCTTTAACCATTTAGA  
GGAAC TGAGACTTCATCTGGAGCAGGAAATGGGCTTTGAAAAATCTTTG  
AGGTTTATGAGAAAATAAAGGCTATTCATGAAGATGAAGATGAAAATATT  
GAAATTTGTTCAAAAATAGTTCAAAAATATTTTGGGAAATGAACATCAGCA  
TCTTTATGCCAAGATTCTTCATTTAGTCATGGCAGATGGAGCCTACCAAG  
AAGATAATGATGAAtaagcggccgc