

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of active NEK2A [1 - 445]**

<b><u>Enzyme description:-</u></b>	NEK2A [1 - 445]
<b><u>Clone number:-</u></b>	DU 406
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system
<b><u>Tag:-</u></b>	N-terminal His(6)
<b><u>Purification method:-</u></b>	Ni <sup>2+</sup> -NTA agarose
<b><u>Expression level:-</u></b>	1-2 mg/L
<b><u>Calculated molecular mass:-</u></b>	52, 684 daltons
<b><u>Purity:-</u></b>	>80 %
<b><u>Activation protocol:-</u></b>	Constitutively active
<b><u>Enzyme storage buffer:-</u></b>	
	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
<b><u>Storage temperature:-</u></b>	-70 °C
<b><u>Assay:-</u></b>	Standard filter binding assay
<b><u>Assay buffer:-</u></b>	
	50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc
<b><u>Substrate:-</u></b>	
NIMA peptide [RFRRSRRMI] Final concentration: 300 µM	Identified from peptide library
<b><u>Specific activity range:-</u></b>	250 – 500 U/mg

*Division of Signal Transduction Therapy*

**Clone Data Sheet**

**NEK2A [1 - 445]**

<b><u>Protein</u></b>	NEK2A [1 - 445]
<b><u>Clone number</u></b>	DU 406
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_002497
<b><u>Tags</u></b>	N-terminal His(6)
<b><u>Baculovirus expressed protein</u></b>	MHHHHHHMPSRAEDYEVLYTIGTGSYGRCQKIRRKSDGKILVWKELDY GSMTEAEKQMLVSEVNLLRELKHPNIVRYYDRIIDRTNTTLYIVMEYC EGGDLASVITKGTKERQYLDEEFVLRVMTQLTLALKECHRRSDGGHTV LHRDLKPANVFLDGKQNVKLGDFGLARILNHDTSF AKTFVGT PYYMSP EQMNRMSYNEKSDIWSLGCLLYELCALMP PFTAFS QKELAGKIREGKF RRIPYRYSDELNEIITRMLNLKDYHRPSVEEILENPLIADLVADEQRR NLERRGRQLGEPEKSQDSSPVLSELKLKEIQLQERERALKAREERLEQ KEQELCVRERLAEDKLARAENLLKNYSLLKERKFLSLASNPELLNLP SVIKKKVHFSGESKENIMRSENSESQLT SKSKCKDLKKRLHAAQLRAQ ALSDIEKNYQLKSRQILGMR
<b><u>Native sequence</u></b>	Amino acids M1 – R445 (end) of human NEK2A. Residue M8 of the fusion protein is equivalent to M1 of NEK2A. The His(6) tag is located at residues 2 - 7 of the fusion protein.
<b><u>Protease cleavage</u></b>	None
<b><u>Cloning sites</u></b>	<i>Nde</i> 1 and <i>Spe</i> 1 of modified pFastBac 1

*Division of Signal Transduction Therapy*

**Nucleotide  
sequence of insert**

ATGCACCATCACCATCACCATATGCCTTCCC GGGCTGAGGACTATGAA  
GTGTTGTACACCATTTGGCACAGGCTCCTACGGCCGCTGCCAGAAGATC  
CGGAGGAAGAGTGATGGCAAGATATTAGTTTGGAAAGAACTTGACTAT  
GGCTCCATGACAGAAGCTGAGAAACAGATGCTTGTCTGAAAGTGAAT  
TTGCTTCGTGAACTGAAACATCCAAACATCGTTTCGTTACTATGATCGG  
ATTATTGACCGGACCAATACAACACTGTACATTGTAATGGAATATTGT  
GAAGGAGGGGATCTGGCTAGTGTAATTACAAAGGGAACCAAGGAAAGG  
CAATACTTAGATGAAGAGTTTGTCTTCGAGTGATGACTCAGTTGACT  
CTGGCCCTGAAGGAATGCCACAGACGAAGTGATGGTGGTCATACCGTA  
TTGCATCGGGATCTGAAACCAGCCAATGTTTTCTGGATGGCAAGCAA  
AACGTCAAGCTTGGGAGACTTTGGGCTAGCTAGAATATTAACCACGAC  
ACGAGTTTTGCAAAAACATTTGTTGGCACACCTTATTACATGTCCTCT  
GAACAAATGAATCGCATGTCCTACAATGAGAAATCAGATATCTGGTCA  
TTGGGCTGCTTGCTGTATGAGTTATGTGCATTAATGCCTCCATTTACA  
GCTTTTAGCCAGAAAGAACTCGCTGGGAAAATCAGAGAAGGCAAATTC  
AGGCGAATTCCATACCGTTACTCTGATGAATTGAATGAAATTATTACG  
AGGATGTTAACTTAAAGGATTACCATCGACCTTCTGTTGAAGAAATT  
CTTGAGAACCCTTTAATAGCAGATTTGGTTGCAGACGAGCAAAGAAGA  
AATCTTGAGAGAAGAGGGCGACAATTAGGAGAGCCAGAAAAATCGCAG  
GATTCCAGCCCTGTATTGAGTGAGCTGAAACTGAAGGAAATTCAGTTA  
CAGGAGCGAGAGCGAGCTCTCAAAGCAAGAGAAGAAAGATTGGAGCAG  
AAAGAACAGGAGCTTTGTGTTTCGTGAGAGACTAGCAGAGGACAAACTG  
GCTAGAGCAGAAAATCTGTTGAAGAACTACAGCTTGCTAAAGGAACGG  
AAGTTCCTGTCTCTGGCAAGTAATCCAGAACTTCTTAATCTTCCATCC  
TCAGTAATTAAGAAGAAAGTTCATTTAGTGGGAAAGTAAAGAGAAC  
ATCATGAGGAGTGAGAATTCTGAGAGTCAGCTCACATCTAAGTCCAAG  
TGCAAGGACCTGAAGAAAAGGCTTCACGCTGCCAGCTGCGGGCTCAA  
GCCCTGTCAGATATTGAGAAAAATTACCAACTGAAAAGCAGACAGATC  
CTGGGCATGCGCtag