Standard Operating Procedure

Preparation of active NEK2A [1 - 445]

Enzyme description:- NEK2A [1 - 445]

Clone number:- DU 406

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Ni^{2+}-NTA agarose

Expression level:- 1-2 mg/L

Calculated molecular mass:- 52, 684 daltons

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,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- -70 °C

Assay:- Standard filter binding assay

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2–mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-

NIMA peptide [RFRRSRRMI] Identified from peptide library
Final concentration: 300 μM

Specific activity range:- 250 – 500 U/mg

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Clone Data Sheet - NEK2A [1 - 445]

Protein	NEK2A [1 - 445]
Clone number	DU 406
Species	Human
Accession number	NM_002497
Tags	N-terminal His(6)
Baculovirus expressed protein	MHHHHHH**MPSRAEDYEVLYTIGTGSYGRCQKIRRKSDGKILVWKELDY GSMTEAEKQMLVSEVNLLRELKHPNIVRYYDRIIDRTNTTLYIVMEYC EGGDLASVITKGTKERQYLDEEFVLRVMTQLTLALKECHRRSDGGHTV LHRDLKPANVFLDGKQNVKLGDFGLARILNHDTSFAKTFVGTPYYMSP EQMNRMSYNEKSDIWSLGCLLYELCALMPPFTAFSQKELAGKIREGKF RRIPYRYSDELNEIITRMLNLKDYHRPSVEEILENPLIADLVADEQRR NLERRGRQLGEPEKSQDSSPVLSELKLKEIQLQERERALKAREERLEQ KEQELCVRERLAEDKLARAENLLKNYSLLKERKFLSLASNPELLNLPS SVIKKKVHFSGESKENIMRSENSESQLTSKSKCKDLKKRLHAAQLRAQ ALSDIEKNYQLKSRQILGMR**
Native sequence	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.
Protease cleavage	None
Cloning sites	*Nde*1 and *Spe*1 of modified pFastBac 1

Nucleotide sequence of insert

ATGCACCATCACCATCACCATATGCCTTCCCGGGCTGAGGACTATGAA
GTGTTGTACACCATTGGCACAGGCTCCTACGGCCGCTGCCAGAAGATC
CGGAGGAAGAGTGATGGCAAGATATTAGTTTGGAAAGAACTTGACTAT
GGCTCCATGACAGAAGCTGAGAAACAGATGCTTGTTTCTGAAGTGAAT
TTGCTTCGTGAACTGAAACATCCAAACATCGTTCGTTACTATGATCGG
ATTATTGACCGGACCAATACAACACTGTACATTGTAATGGAATATTGT
GAAGGAGGGGATCTGGCTAGTGTAATTACAAAGGGAACCAAGGAAAGG
CAATACTTAGATGAAGAGTTTGTTCTTCGAGTGATGACTCAGTTGACT
CTGGCCCTGAAGGAATGCCACAGACGAAGTGATGGTGGTCATACCGTA
TTGCATCGGGATCTGAAACCAGCCAATGTTTTCCTGGATGGCAAGCAA
AACGTCAAGCTTGGAGACTTTGGGCTAGCTAGAATATTAAACCACGAC
ACGAGTTTTGCAAAAACATTTGTTGGCACACCTTATTACATGTCTCCT
GAACAAATGAATCGCATGTCCTACAATGAGAAATCAGATATCTGGTCA
TTGGGCTGCTTGCTGTATGAGTTATGTGCATTAATGCCTCCATTTACA
GCTTTTAGCCAGAAAGAACTCGCTGGGAAAATCAGAGAAGGCAAATTC
AGGCGAATTCCATACCGTTACTCTGATGAATTGAATGAAATTATTACG
AGGATGTTAAACTTAAAGGATTACCATCGACCTTCTGTTGAAGAAATT
CTTGAGAACCCTTTAATAGCAGATTTGGTTGCAGACGAGCAAAGAAGA
AATCTTGAGAGAAGAGGGCGACAATTAGGAGAGCCAGAAAAATCGCAG
GATTCCAGCCCTGTATTGAGTGAGCTGAAACTGAAGGAAATTCAGTTA
CAGGAGCGAGAGCGAGCTCTCAAAGCAAGAGAAGAAAGATTGGAGCAG
AAAGAACAGGAGCTTTGTGTTCGTGAGAGACTAGCAGAGGACAAACTG
GCTAGAGCAGAAAATCTGTTGAAGAACTACAGCTTGCTAAAGGAACGG
AAGTTCCTGTCTCTGGCAAGTAATCCAGAACTTCTTAATCTTCCATCC
TCAGTAATTAAGAAGAAAGTTCATTTCAGTGGGGAAAGTAAAGAGAAC
ATCATGAGGAGTGAGAATTCTGAGAGTCAGCTCACATCTAAGTCCAAG
TGCAAGGACCTGAAGAAAAGGCTTCACGCTGCCCAGCTGCGGGCTCAA
GCCCTGTCAGATATTGAGAAAAATTACCAACTGAAAAGCAGACAGATC
CTGGGCATGCGCtag