Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active NEK6 [8 - 313]

Enzyme description:- NEK6 [8 - 313]

Clone number:- DU 635

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6) and FLAG

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

Expression level:- 1-2 mg/L

Calculated molecular mass:- 41, 616 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:-
FLAKSFGPNRAYKK Identified from peptide library
Final concentration: 300 µM

Specific activity range:- 50 - 100 U/mg

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Clone Data Sheet

NEK6 [8 - 313]

Protein	NEK6 [8 – 313]
Clone number	DU 635
Species	Human
Accession number	NM_014397
Tags	N-terminal His(6) and FLAG (DYKDDDDK)
Baculovirus-expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGIRNSKAYVDELTSATMDYK DDDDKAGQPGH**MPHGGSSNNLCHTLGPVHPPDPQRHPNTLSFRCSL ADFQIEKKIGRGQFSEVYKATCLLDRKTVALKKVQIFEMMDAKARQ DCVKEIGLLKQLNHPNIIKYLDSFIEDNELNIVLELADAGDLSQMI KYFKKQKRLIPERTVWKYFVQLCSAVEHMHSRRVMHRDIKPANVFI TATGVVKLGDLGLGRFFSSETTAAHSLVGTPYYMSPERIHENGYNF KSDIWSLGCLLYEMAALQSPFYGDKMNLFSLCQKIEQCDYPPLPGE HYSEKLRELVSMCICPDPHQRPDIGYVHQVAKQMHIWMSST**
Native sequence	Amino acids M8 – T313 (end) of human NEK6. Residue M58 of the fusion protein is equivalent to M8 of the native enzyme. The His(6) tag is located at residues 5 - 10 and the FLAG tag is located at residues 43 - 51.
Protease cleavage	rTEV cleavage site (ENLYFQG) residues 18 - 24
Cloning sites	*Spe*1 site of pFastBAC HTc

Nucleotide sequence of insert

ATGCCCCATGGAGGGAGTTCCAACAACCTCTGCCACACCCTGGGGCCTG
TGCATCCTCCTGACCCACAGAGGCATCCCAACACGCTGTCTTTTCGCTG
CTCGCTGGCGGACTTCCAGATCGAAAAGAAGATAGGCCGAGGACAGTTC
AGCGAGGTGTACAAGGCCACCTGCCTGCTGGACAGGAAGACAGTGGCTC
TGAAGAAGGTGCAGATCTTTGAGATGATGGACGCCAAGGCGAGGCAGGA
CTGTGTCAAGGAGATCGGCCTCTTGAAGCAACTGAACCACCCAAATATC
ATCAAGTATTTGGACTCGTTTATCGAAGACAACGAGCTGAACATTGTGC
TGGAGTTGGCTGACGCAGGGGACCTCTCGCAGATGATCAAGTACTTTAA
GAAGCAGAAGCGGCTCATCCCGGAGAGGACAGTATGGAAGTACTTTGTG
CAGCTGTGCAGCGCCGTGGAGCACATGCATTCACGCCGGGTGATGCACC
GAGACATCAAGCCTGCCAACGTGTTCATCACAGCCACGGGCGTCGTGAA
GCTCGGTGACCTTGGTCTGGGCCGCTTCTTCAGCTCTGAGACCACCGCA
GCCCACTCCCTAGTGGGGACGCCCTACTACATGTCACCGGAGAGGATCC
ATGAGAACGGCTACAACTTCAAGTCCGACATCTGGTCCCTGGGCTGTCT
GCTGTACGAGATGGCAGCCCTCCAGAGCCCCTTCTATGGAGATAAGATG
AATCTCTTCTCCCTGTGCCAGAAGATCGAGCAGTGTGACTACCCCCCAC
TCCCCGGGGAGCACTACTCCGAGAAGTTACGAGAACTGGTCAGCATGTG
CATCTGCCCTGACCCCCACCAGAGACCTGACATCGGATACGTGCACCAG
GTGGCCAAGCAGATGCACATCTGGATGTCCAGCACC