

Standard Operating Procedure

Preparation of active NEK6

Enzyme description:-	Active NEK6
Source:-	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system (BEVS)/Insect cells
<u>Tag:-</u>	His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose.
<u>Expression level:-</u>	1-2mg/L
<u>Molecular mass:-</u>	42 kDa by SDS-PAGE
<u>Purity:-</u>	>80%
<u>Contaminants:-</u>	Some minor contaminating proteins as judged by Novex gel.
<u>Activation protocol:-</u>	None- constitutively active
<u>Enzyme storage buffer:-</u>	50 mM Tris/HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % β -mercaptoethanol, 0.02% Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.
<u>Storage temperature:-</u>	Aliquot, snap freeze and store at -70°C.

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CLONE DATA SHEET – human NEK6

<u>Protein</u>	Human NEK6
<u>Accession number</u>	XM_044814
<u>Tags</u>	His(6) and FLAG (DYKDDDDK) amino terminal
<u>Baculovirus-expressed protein</u>	MSYYHHHHHDYDIPTTENLYFQGAMGIRNSKAYVDELT SATMDYKDDDDKAGQPGHMPHGGSSNNLCHTLGVPVHP PDPQRHPNTLSFRCSLADFQIEKKIGRGQFSEVYKATCLL DRKTVALKKVQIFEMMDAKARQDCVKEIGLLKQLNHP NIIKYLDSFIEDNELNIVLELADAGDLSQMIKYFKKQKRL IPERTVWKYFVQLCSAVEHMHSRRVMHRDIKPANVFIT ATGVVKLGLDLGLGRFFSSETTAAHSLVGTPTYMSPERIH ENGYNFKSDIWSLGCLLYEMAALQSPFYGDKMNLFSLC QKIEQCDYPLPGEHYSEKLRRELVSMCICPDPHQRPDIGY VHQVAKQMHIWMSST
<u>Native sequence</u>	Residue 58 of the His/FLAG tagged protein is equivalent to Met 1 of NEK6. The His (6) tag is located at residues 5 -10 of the tagged protein, while the FLAG tag is located at residues 43-51 of the tagged protein.
<u>Protease cleavage site</u>	rTEV cleavage site (ENLYF) residues 18-22.

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Cloning sites

*Spe*1 site of pFastBAC HTc

ORF

In baculovirus

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ATGCCCCATGGAGGGAGTTCCAACAACCTCTGCCACACCCTGGGGCCTG
TGCATCCTCCTGACCCACAGAGGCATCCCAACACGCTGTCTTTTCGCTG
CTCGCTGGCGGACTTCCAGATCGAAAAGAAGATAGGCCGAGGACAGTTC
AGCGAGGTGTACAAGGCCACCTGCCTGCTGGACAGGAAGACAGTGGCTC
TGAAGAAGGTGCAGATCTTTGAGATGATGGACGCCAAGGCCGAGGCAGGA
CTGTGTCAAGGAGATCGGCCTCTTGAAGCAACTGAACCACCCAAATATC
ATCAAGTATTTGGACTCGTTTATCGAAGACAACGAGCTGAACATTGTGC
TGGAGTTGGCTGACGCAGGGGACCTCTCGCAGATGATCAAGTACTTTAA
GAAGCAGAAGCGGCTCATCCCGGAGAGGACAGTATGGAAGTACTTTGTG
CAGCTGTGCAGCGCCGTGGAGCACATGCATTCACGCCGGGTGATGCACC
GAGACATCAAGCCTGCCAACGTGTTTCATCACAGCCACGGGCGTCGTGAA
GCTCGGTGACCTTGGTCTGGGCCGCTTCTTTCAGCTCTGAGACCACCGCA
GCCACTCCCTAGTGGGGACGCCCTACTACATGTCACCGGAGAGGATCC
ATGAGAACGGCTACAACCTCAAGTCCGACATCTGGTCCCTGGGCTGTCT
GCTGTACGAGATGGCAGCCCTCCAGAGCCCCTTCTATGGAGATAAGATG
AATCTCTTCTCCCTGTGCCAGAAGATCGAGCAGTGTGACTACCCCCAC
TCCCCGGGAGCACTACTCCGAGAAGTTACGAGAACTGGTCAGCATGTG
CATCTGCCCTGACCCCCACCAGAGACCTGACATCGGATACGTGCACCAG
GTGGCCAAGCAGATGCACATCTGGATGTCCAGCACC
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