MRCPPU Reagents and Services

Standard Operating Procedure

Preparation of active NEK6 [1 – 313]

Enzyme description:- NEK6 [1 - 313]

Clone number:- DU 71996

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal GST

Purification method:- GSH Agarose

Calculated molecular mass:-

Monoisotopic	62,870.67 daltons
Average Mass	62,911.88 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.73

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, 0.5 mM TCEP

Storage temperature:- -70 °C

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 5 mM MgAc

Substrate:-
FLAKSFGSPNRAYKK Final concentration: 300 uM

Clone Data Sheet

NEK6 [1 - 313]

Protein	NEK6 [1 - 313]
Clone number	DU 71996
Species	Human
Accession number	NM_014397
Tags	N-terminal GST

Baculovirus expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNK
KFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKE
RAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKM
FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL
VCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD
LEVLFQGPLGSPEF**MAGQPGHMPHGGSSNNLCHTLGPVHPPDPQ
RHPNTLSFRCSLADFQIEKKIGRGQFSEVYKATCLLDRKTVALK
KVQIFEMMDAKARQDCVKEIGLLKQLNHPNIIKYLDSFIEDNEL
NIVLELADAGDLSQMIKYFKKQKRLIPERTVWKYFVQLCSAVEH
MHSRRVMHRDIKPANVFITATGVVKLGDLGLGRFFSSETTAAHS
LVGTPYYMSPERIHENGYNFKSDIWSLGCLLYEMAALQSPFYGD
KMNLFSLCQKIEQCDYPPLPGEHYSEKLRELVSMCICPDPHQRP
DIGYVHQVAKQMHIWMSST**

Native sequence Amino acids M1 – T313 (end residue) of human NEK6. Residue M235 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 *Eco*R1 and *Not*1sites of pFastBac Dual.