MRCPPU Reagents and Services

Standard Operating Procedure

Preparation of active NEK9 [1 - 979]

Enzyme description:- NEK9 [1 - 979]

Clone number:- DU 71945

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal GST

Purification method:- GSH Agarose

Calculated molecular mass:-
Monoisotopic 133,906.85 daltons
Average Mass 133,992.63 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.55

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 MnCl

Substrate:-
Myelin Basic Protein Final concentration: 1 mg/ml

Clone Data Sheet

NEK9 [1 - 979]

Protein	NEK9 [1 - 979]
Clone number	DU 71945
Species	Human
Accession number	NM_033116.6
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNK KFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKE RAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKM FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL VCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD LEVLFQGPLGS**MSVLGEYERHCDSINSDFGSESGGCGDSSPGPS** **ASQGPRAGGGAAEQEELHYIPIRVLGRGAFGEATLYRRTEDDSL** **VVWKEVDLTRLSEKERRDALNEIVILALLQHDNIIAYYNHFMDN** **TTLLIELEYCNGGNLYDKILRQKDKLFEEEMVVWYLFQIVSAVS** **CIHKAGILHRDIKTLNIFLTKANLIKLGDYGLAKKLNSEYSMAE** **TLVGTPYYMSPELCQGVKYNFKSDIWAVGCVIFELLTLKRTFDA** **TNPLNLCVKIVQGIRAMEVDSSQYSLELIQMVHSCLDQDPEQRP** **TADELLDRPLLRKRRREMEEKVTLLNAPTKRPRSSTVTEAPIAV** **VTSRTSEVYVWGGGKSTPQKLDVIKSGCSARQVCAGNTHFAVVT** **VEKELYTWVNMQGGTKLHGQLGHGDKASYRQPKHVEKLQGKAIR** **QVSCGDDFTVCVTDEGQLYAFGSDYYGCMGVDKVAGPEVLEPMQ** **LNFFLSNPVEQVSCGDNHVVVLTRNKEVYSWGCGEYGRLGLDSE** **EDYYTPQKVDVPKALIIVAVQCGCDGTFLLTQSGKVLACGLNEF** **NKLGLNQCMSGIINHEAYHEVPYTTSFTLAKQLSFYKIRTIAPG** **KTHTAAIDERGRLLTFGCNKCGQLGVGNYKKRLGINLLGGPLGG** **KQVIRVSCGDEFTIAATDDNHIFAWGNGGNGRLAMTPTERPHGS** **DICTSWPRPIFGSLHHVPDLSCRGWHTILIVEKVLNSKTIRSNS** **SGLSIGTVFQSSSPGGGGGGGGGEEEDSQQESETPDPSGGFRGT** **MEADRGMEGLISPTEAMGNSNGASSSCPGWLRKELENAEFIPMP** **DSPSPLSAAFSESEKDTLPYEELQGLKVASEAPLEHKPQVEASS** **PRLNPAVTCAGKGTPLTPPACACSSLQVEVERLQGLVLKCLAEQ** **QKLQQENLQIFTQLQKLNKKLEGGQQVGMHSKGTQTAKEEMEMD** **PKPDLDSDSWCLLGTDSCRPSL**

Native sequence	Amino acids M1 – L979 (end residue) of human NEK9. Residue M232 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	*Bam*H1 and *Not*1sites of pFastBac Dual.

Nucleotide sequence of insert

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TCGGACTTTGGGAGCGAGTCCGGGGGTTGCGGGGACTCGAGTCCG
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