Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active NEK7 [2 - 302]

Enzyme description:- NEK7 [2 - 302]

Clone number:- DU 631

Source:- Recombinant

Expression system:- Baculovirus expression vector system

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

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

Expression level:- 1-2 mg/L

Calculated molecular mass:- 39, 179 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:- 40 – 80 U/mg

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Clone Data Sheet

NEK7 [2 - 302]

Protein	NEK7 [2 – 302]
Clone number	DU 631
Species	Human
Accession number	NM_133494
Tags	N-terminal His(6) and HA (YPYDVPDYA)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGSATMYPYDVPDYA**DEQSQG MQGPPVPQFQPQKALRPDMGYNTLANFRIEKKIGRGQFSEVYRAAC LLDGVPVALKKVQIFDLMDAKARADCIKEIDLLKQLNHPNVIKYYA SFIEDNELNIVLELADAGDLSRMIKHFKKQKRLIPERTVWKYFVQL CSALEHMHSRRVMHRDIKPPNVFITATGVVKLGDLGLGRFFSSKTT AAHSLVGTPYYMSPERIHENGYNFKSDIWSLGCLLYEMAALQSPFY GDKMNLYSLCKKIEQCDYPPLPSDHYSEELRQLVNMCINPDPEKRP DVTYVYDVAKRMHACTASS**
Native sequence	Amino acids D2 – S302 (end) of human NEK7. Residue D41 of the fusion protein is equivalent to D2 of the native enzyme. The His(6) tag is located at residues 5 -10 and the HA tag (YPYDVPDYA) is located at residues 32 – 40. The following amino acid substitutions are present: A – P, where A165 of the native sequence is P204 of the fusion protein.
Protease cleavage	rTEV cleavage site (ENLYFQG) residues 18 - 24.
Cloning sites	*BamH*1 site of pFastBAC HTb

Nucleotide sequence of insert

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGA
CCGAAAACCTGTATTTTCAGGGCGCCATGGGATCCGCCACCATGTACCC
ATACGATGTGCCAGATTACGCCGATGAGCAATCACAAGGAATGCAAGGG
CCACCTGTTCCTCAGTTCCAACCACAGAAGGCCTTACGACCGGATATGG
GCTATAATACATTAGCCAACTTTCGAATAGAAAAGAAAATTGGTCGCGG
ACAATTTAGTGAAGTTTATAGAGCAGCCTGTCTCTTGGATGGAGTACCA
GTAGCTTTAAAAAAAGTGCAGATATTTGATTTAATGGATGCCAAAGCAC
GTGCTGATTGCATCAAAGAAATAGATCTTCTTAAGCAACTCAACCATCC
AAATGTAATAAAATATTATGCATCATTCATTGAAGATAATGAACTAAAC
ATAGTTTTGGAACTAGCAGATGCTGGCGACCTATCCAGAATGATCAAGC
ATTTTAAGAAGCAAAAGAGGCTAATTCCTGAAAGAACTGTTTGGAAGTA
TTTTGTTCAGCTTTGCAGTGCATTGGAACACATGCATTCTCGAAGAGTC
ATGCATAGAGATATAAAACCACCTAATGTGTTCATTACAGCCACTGGGG
TGGTAAAACTTGGAGATCTTGGGCTTGGCCGGTTTTTCAGCTCAAAAAC
CACAGCTGCACATTCTTTAGTTGGTACGCCTTATTACATGTCTCCAGAG
AGAATACATGAAAATGGATACAACTTCAAATCTGACATCTGGTCTCTTG
GCTGTCTACTATATGAGATGGCTGCATTACAAAGTCCTTTCTATGGTGA
CAAAATGAATTTATACTCACTGTGTAAGAAGATAGAACAGTGTGACTAC
CCACCTCTTCCTTCAGATCACTATTCAGAAGAACTCCGACAGTTAGTTA
ATATGTGCATCAACCCAGATCCAGAGAAGCGACCAGACGTCACCTATGT
TTATGACGTAGCAAAGAGGATGCATGCATGCACTGCAAGCAGCtaa