

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active NUAk1 [2 - 660]

<u>Enzyme description:-</u>	NUAK1 [2 – 660]
<u>Clone number:-</u>	DU 1271
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6) tag
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	1 mg/L
<u>Calculated molecular mass:-</u>	77, 841 daltons
<u>Purity:-</u>	75 %
<u>Activation protocol:-</u>	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 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.
<u>Storage temperature:-</u>	-70 °C [Long term stability to be determined]
<u>Assay:-</u>	Standard filter binding assay
<u>Assay Buffer:-</u>	
	50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc
<u>Substrate:-</u>	
	Generic peptide [KKLNRTL S FAEPG] Final concentration: 300 μM
<u>Specific activity range:-</u>	To be determined

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Clone Data Sheet

NUAK1 [2 - 660]

<u>Protein</u>	NUAK1 [2 – 660]
<u>Clone Number</u>	DU 1271
<u>Species</u>	Human
<u>Accession number</u>	NM_014840
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MSYYHHHHHDYDIPTTENLYFQGAMDPEFEGAAAPVAGDRPDLGLGAP GSPREAVAGATAALEPRKPHGVKRHHHKHNLKHRYELQETLGKGTYGKV KRATERFSGRVVAIKSIRKDKIKDEQDMVHIRREIEIMSSLNHPHIISI YEVFENKDKIVIIMEYASKGELYDYISERRRLSERETRHFFRQIVSAVH YCHKNGVVHRDLKLENILLDDNCNIKIADFGLSNLYQKDKFLQTFCGSP LYASPEIVNGRPYRGPEVDSWALGVLLYTLVYGTMPFDGFDHKNLIRQI SSGEYREPTQPSDARGLIRWMLMVNPDRRATIEDIANHWWVNWGYKSSV CDCDALHDSSEPLLARIIDWHHRSTGLQADTEAKMKGLAKPTTSEVMLE RQRSLKSKKENDFAQSGQDAVPESPSKLSKRPKILKRSNSEHRSH STGFIEGVGPALPSTFKMEQDLCRTGVLLPSSPEAEVPGKLSPKQSAT MPKKGILKKTQQRESGYSSPERSESSELLDSNDVMGSSIPSPSPDPA RVTSHSLSCRRKGILKHSSKYSAGTMDPALVSPEMPTLESLEPGVPAE GLSRSYSRPSVVISDDSVLSSDSFDLLDLQENRPARQRIRSCVSAENFL QIQDFEGLQNRPRPQYLKRYRNRLADSSFLLTDMDDVTQVYKQALEIC SKLN
<u>Native sequence</u>	Amino acids E2 – N660 (end) of human NUAK1. Residue E31 of fusion protein is equivalent to E2 of the native enzyme. The His(6) tag is located at residues 5 – 10.
<u>Protease cleavage</u>	rTEV (ENLYFQG) residues 18 - 24
<u>Cloning sites</u>	<i>Eco</i> R1 site of pFastBAC HTa

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**Nucleotide
sequence of
insert**

gaattcGAAGGGGCCCGCGCCTGTGGCGGGGACCGCCCCGACTTGG
GGCTGGGGGCGCCGGGCTCTCCCCGAGAGGCGGTGGCGGGGGCGACTGC
AGCCCTGGAGCCCAGGAAGCCGCACGGGGTGAAGCGGCATCACCAAG
CACAACTTGAAGCACCGCTACGAGCTGCAGGAGACCCTGGGCAAAGGCA
CCTACGGCAAAGTCAAGCGGGCCACCGAGAGGTTTTCTGGCCGAGTGGT
TGCTATAAAATCCATTTCGTAAGGACAAAATTAAGGATGAACAAGACATG
GTTACATCAGACGAGAGATTGAGATCATGTCATCTCTCAACCATCCTC
ATATCATCAGTATTTATGAAGTGTTTGAGAACAAAGATAAGATTGTGAT
CATCATGGAATATGCCAGCAAAGGGGAGCTGTACGATTACATCAGTGAG
CGGCGACGCCTCAGTGAGAGGGAGACCCGGCACTTCTTCCGGCAGATCG
TCTCTGCTGTGCAC'TATTGTACAAGAACGGTGTGGTCCACCGGGACTT
GAAGCTGGAAAATATACTGCTCGATGACAACCTGCAATATTAAGATTGCT
GACTTTGGGCTTTCCAACCTGTACCAGAAGGATAAGTTCCTTACAAACGT
TTTGTGGGAGTCCACTCTATGCATCTCCTGAGATTGTCAATGGGAGACC
TTACCGAGGGCCAGAGGTGGACAGCTGGGCCCTGGGTGTGTTGCTTTAC
ACTCTTGTTTATGGAACAATGCCCTTCGATGGTTTCGATCACAAAACC
TCATTCGGCAAATCAGCAGCGGAGAGTACCGGGAGCCAACACAGCCCTC
AGATGCTCGAGGACTCATACGGTGGATGCTGATGGTGAACCCCGATCGC
CGGGCCACTATTGAGGACATTGCCAACCCTGGTGGGTGAACCTGGGGCT
ATAAGAGCAGCGTGTGTGACTGTGATGCCCTCCATGACTCTGAGTCCCC
ACTCCTGGCTCGGATCATTGACTGGCACCACCGTTCCACAGGGCTGCAG
GCTGACACCCGAAGCCAAAATGAAGGGCCTGGCCAAACCCACGACCTCTG
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TGACTTTGCTCAGTCTGGTCAGGATGCAGTGCCTGAAAGCCCATCCAAG
TTGAGTTCTAAGAGGCCCAAGGGGATCCTGAAGAAGCGAAGCAACAGCG
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CTTACCCTCTACTTTCAAGATGGAGCAGGACTTGTGCAGGACTGGCGTG
CTCCTCCCAAGCTCACAGAGGCAGAGGTGCCGGGAAAACCTCAGCCCCA
AGCAGTCGGCCACGATGCCCAAGAAAGGCATCTTGAAAAAGACCAGCA
GAGAGAATCAGGTTACTACTCTTCCCCAGAGCGCAGTGAGTCTTCGGAG
CTGTTGGACAGTAATGATGTGATGGGCAGCAGCATCCCCCTCCCCAGCC
CCCCGGACCCAGCCAGGGTAACCTCCACAGCCTCTCCTGCCGGAGGAA
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GCCCTGGTCAGCCCTGAAATGCCACACTGGAATCCCTGTGAGAGCCTG
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CATCAGCGATGACAGCGTGTGTCAGCGACTCTTTTGACTTGCTGGAT
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CAGAAAACCTCCTCCAGATCCAGGACTTTGAGGGGCTCCAGAACCGGCC
CCGGCCCCAGTACCTGAAGCGGTACCGGAACCGGCTGGCAGACAGCAGC
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CGCTGGAGATCTGCAGCAAGCTCAACTaggaattc