

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active NUA1 [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>	
Monoisotopic	77, 841.32 daltons
Average Mass	77, 889.99 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	8.58
<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	

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Substrate:-

ALNRTSSDSALHRRR

Residues 165 – 176 of TORC2 plus three Arginine residues at C-terminus

Final concentration: 300 μ M

Specific activity range:-

To be determined

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Clone Data Sheet

NUAK1 [2 - 660]

Protein NUA1 [2 – 660]

Clone Number DU 1271

Species Human

Accession number NM_014840

Tags N-terminal His(6)

Baculovirus expressed protein MSYYHHHHHDYDIPTTENLYFQGAMDPEFEGAAAPVAGDRPDLGLGAP
GSPREAVAGATAALEPRKPHGVKRHHHKHNLKHRYELQETLGKGTYGKV
KRATERFSGRVVAIKSIRKDKIKDEQDMVHIRREIEIMSSLNHPHIISI
YEVFENKDKIVIIMEYASKGELYDYISERRRLSERETRHFFRQIVSAVH
YCHKNGVVHRDLKLENILLDDNCNIKIADFGLSNLYQKDKFLQTF CGSP
LYASPEIVNGRPYRGPEVDSWALGVLLYTLVYGTMPPFDGFDHKNLIRQI
SSGEYREPTQPSDARGLIRWMLMVNPDRRATIEDIANHWWVNWGYKSSV
CDCDALHDSSEPLLARIIDWHHRSTGLQADTEAKMKGLAKPTTSEVMLE
RQRLKSKSKKENDFAQSGQDAVPESPSKLSSKRPKGILKKRSNSEHRSH
STGFIEGVGPALPSTFKMEQDLCRTGVLLPSSPEAEVPGKLSPKQSAT
MPKKGILKKTQQRESGYSSPERSESSELDSNDVMGSSIPSPSPDPA
RVTSHSLSCRRKGILKHSSKYSAGTMDPALVSPEMPTLESLEPGVPAE
GLSRSYSRPSSVISDDSVLSSDSFDLLDLQENRPARQIRSCVSAENFL
QIQDFEGLQNRPRPQYLKRYRNRLADSSFSLLTMDDDVTQVYKQALEIC
SKLN

Native sequence Amino acids E2 – N660 (end) of human NUA1.
Residue E31 of fusion protein is equivalent to E2 of the native enzyme.
The His(6) tag is located at residues 5 – 10.

Protease cleavage rTEV (ENLYFQG) residues 18 - 24

Cloning sites EcoR1 site of pFastBAC HTa

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Nucleotide sequence of insert

gaattcGAAGGGGCCGCCGCGCCTGTGGCGGGGGACCGCCCCGACTTGG
GGCTGGGGGCGCGCGGGCTCTCCCCGAGAGGCGGTGGCGGGGGCGACTGC
AGCCCTGGAGCCCAGGAAGCCGCACGGGGTGAAGCGGCATCACCACAAG
CACAACCTGAAGCACCGCTACGAGCTGCAGGAGACCCTGGGCAAAGGCA
CCTACGGCAAAGTCAAGCGGGCCACCGAGAGGTTTTCTGGCCGAGTGGT
TGCTATAAAATCCATTTCGTAAGGACAAAATTAAGGATGAACAAGACATG
GTTACATCAGACGAGAGATTGAGATCATGTCATCTCTCAACCATCCTC
ATATCATCAGTATTTATGAAGTGTTTGAGAACAAAGATAAGATTGTGAT
CATCATGGAATATGCCAGCAAAGGGGAGCTGTACGATTACATCAGTGAG
CGGCGACGCCTCAGTGAGAGGGAGACCCGGCAGTTCTTCCGGCAGATCG
TCTCTGCTGTGCACTATTGTACAAGAACGGTGTGGTCCACCGGGACTT
GAAGCTGGAAAATATACTGCTCGATGACAACCTGCAATATTAAGATTGCT
GACTTTGGGCTTTCCAACCTGTACCAGAAGGATAAGTTCTTACAAACGT
TTTGTGGGAGTCCACTCTATGCATCTCCTGAGATTGTCAATGGGAGACC
TTACCGAGGGCCAGAGGTGGACAGCTGGGCCCTGGGTGTGTTGCTTTAC
ACTCTTGTTTATGGAACAATGCCCTTCGATGGTTTCGATCACAAAACC
TCATTTCGGCAAATCAGCAGCGGAGAGTACCGGGAGCCAACACAGCCCTC
AGATGCTCGAGGACTCATACGGTGGATGCTGATGGTGAACCCCGATCGC
CGGGCCACTATTGAGGACATTGCCAACCCTGGTGGGTGAACGGGGCT
ATAAGAGCAGCGTGTGTGACTGTGATGCCCTCCATGACTCTGAGTCCCC
ACTCCTGGCTCGGATCATTGACTGGCACCACCGTTCCACAGGGCTGCAG
GCTGACACCGAAGCCAAAATGAAGGGCCTGGCCAAACCCACGACCTCTG
AGGTCATGCTAGAGCGGCAGCGGTCTGCTGAAGAAATCCAAGAAAGAGAA
TGACTTTGCTCAGTCTGGTCAGGATGCAGTGCCCTGAAAGCCCATCCAAG
TTGAGTTCTAAGAGGCCCAAGGGGATCCTGAAGAAGCGAAGCAACAGCG
AGCATCGCTCTCACAGCACTGGCTTCATTGAAGGTGTAGTTGGTCTCTGC
CTTACCCTCTACTTTCAAGATGGAGCAGGACTTGTGCAGGACTGGCGTG
CTCCTCCCAAGCTCACCAGAGGCAGAGGTGCCGGGAAAACCTCAGCCCCA
AGCAGTCGGCCACGATGCCCAAGAAAGGCATCTTGAAAAAGACCCAGCA
GAGAGAATCAGGTTACTACTCTTCCCCAGAGCGCAGTGAGTCTTCGGAG
CTGTTGGACAGTAATGATGTGATGGGCAGCAGCATCCCCCTCCCCAGCC
CCCCGGACCCAGCCAGGGTAACCTCCACAGCCTCTCCTGCCGGAGGAA
GGGCATCTTGAAACACAGCAGCAAATACTCAGCGGGCACCATGGACCCA
GCCCTGGTCAGCCCTGAAATGCCACACTGGAATCCCTGTCAGAGCCTG
GTGTCCCTGCCGAGGGCCTCTCCCGGAGCTACAGCCGCCCTTCCAGTGT
CATCAGCGATGACAGCGTGCTGTCCAGCGACTCTTTTGACTTGCTGGAT
TTGCAGGAGAATCGCCCTGCCCGCCAGCGCATCCGCAGCTGCGTCTCTG
CAGAAAACCTCCTCCAGATCCAGGACTTTGAGGGGCTCCAGAACCGGCC
CCGGCCCCAGTACCTGAAGCGGTACCGGAACCGGCTGGCAGACAGCAGC
TTCTCCCTCCTCACAGACATGGATGATGTGACTCAGGTCTACAAGCAAG
CGCTGGAGATCTGCAGCAAGCTCAACtaggaattc