Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active ULK3 [1 - 492]

Enzyme description:- ULK3 [1 – 492]

Clone number:- DU 43476

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 80, 217.43 daltons
Average Mass 80, 268.66 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.38

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM MgAc

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

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Clone Data Sheet

ULK3 [1 - 492]

Protein	ULK3 [1 - 492]
Clone number	DU 43476
Species	Human
Accession number	Q6PHR2.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MAGPGWGPP** **RLDGFILTERLGSGTYATVYKAYAKKDTREVVAIKCVAKKSLNKASVE** **NLLTEIEILKGIRHPHIVQLKDFQWDSDNIYLIMEFCAGGDLSRFIHT** **RRILPEKVARVFMQQLASALQFLHERNISHLDLKPQNILLSSLEKPHL** **KLADFGFAQHMSPWDEKHVLRGSPLYMAPEMVCQRQYDARVDLWSMGV** **ILYEALFGQPPFASRSFSELEEKIRSNRVIELPLRPLLSRDCRDLLQR** **LLERDPSRRISFQDFFAHPWVDLEHMPSGESLGRATALVVQAVKKDQE** **GDSAAALSLYCKALDFFVPALHYEVDAQRKEAIKAKVGQYVSRAEELK** **AIVSSSNQALLRQGTSARDLLREMARDKPRLLAALEVASAAMAKEEAA** **GGEQDALDLYQHSLGELLLLLAAEPPGRRRELLHTEVQNLMARAEYLK** **EQVKMRESRWEADTLDKEGLSESVRSSCTLQ**
Native sequence	Amino acids M1 – Q472 (end) of human ULK3. 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 site (LEVLFQGP) residues 221 – 228
Cloning sites	*Bam*H1 and *Not*1 sites of pFastBac GST 6P1

Nucleotide sequence of insert

ggatccATGGCGGGGCCCGGCTGGGGTCCCCCGCGCCTGGACGGCTTC
ATCCTCACCGAGCGCCTGGGCAGCGGCACGTACGCCACGGTGTACAAG
GCCTACGCCAAGAAGGACACTCGTGAAGTGGTAGCCATAAAGTGTGTA
GCCAAGAAAAGTCTGAACAAGGCATCGGTGGAGAACCTCCTCACGGAG
ATTGAGATCCTCAAGGGCATTCGACATCCCCACATTGTGCAGCTGAAA
GACTTTCAGTGGGACAGTGACAATATCTACCTCATCATGGAGTTTTGC
GCAGGGGGCGACCTGTCTCGCTTCATCCATACCCGCAGGATTCTGCCT
GAGAAGGTGGCGCGTGTCTTCATGCAGCAATTAGCTAGCGCCCTGCAA
TTCCTGCATGAACGGAATATCTCTCACCTGGATCTGAAGCCACAGAAC
ATTCTACTGAGCTCCTTGGAGAAGCCCCACCTAAAACTGGCAGACTTT
GGTTTCGCACAACACATGTCCCCGTGGGATGAGAAGCACGTGCTCCGT
GGCTCCCCCCTCTACATGGCCCCCGAGATGGTGTGCCAGCGGCAGTAT
GACGCCCGCGTGGACCTCTGGTCCATGGGGGTCATCCTGTATGAAGCC
CTCTTCGGGCAGCCCCCCTTTGCCTCCAGGTCGTTCTCGGAGCTGGAA
GAGAAGATCCGTAGCAACCGGGTCATCGAGCTCCCCTTGCGGCCCCTG
CTCTCCCGAGACTGCCGGGACCTACTGCAGCGGCTCCTGGAGCGGGAC
CCCAGCCGTCGCATCTCCTTCCAGGACTTTTTTGCGCACCCCTGGGTG
GACCTGGAGCACATGCCCAGTGGGGAGAGTCTGGGGCGAGCAACCGCC
CTGGTGGTGCAGGCTGTGAAGAAAGACCAGGAGGGGGATTCAGCAGCT
GCCTTATCACTCTACTGCAAGGCTCTGGACTTCTTTGTACCTGCCCTG
CACTATGAAGTGGATGCCCAGCGGAAGGAGGCAATTAAGGCAAAGGTG
GGGCAGTACGTGTCCCGGGCTGAGGAGCTCAAGGCCATCGTCTCCTCT
TCCAATCAGGCCCTGCTGAGGCAGGGGACCTCTGCCCGAGACCTGCTC
AGAGAGATGGCCCGGGACAAGCCACGCCTCCTAGCTGCCCTGGAAGTG
GCTTCAGCTGCCATGGCCAAGGAGGAGGCCGCCGGCGGGGAGCAGGAT
GCCCTGGACCTGTACCAGCACAGCCTGGGGGAGCTACTGCTGTTGCTG
GCAGCGGAGCCCCCGGGCCGGAGGCGGGAGCTGCTTCACACTGAGGTT
CAGAACCTCATGGCCCGAGCTGAATACTTGAAGGAGCAGGTCAAGATG
AGGGAATCTCGCTGGGAAGCTGACACCCTGGACAAAGAGGGACTGTCG
GAATCTGTTCGTAGCTCTTGCACCCTTCAGtgagcggccgc