

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active ULK1 [2 – 1050]

<u>Enzyme description:-</u>	ULK1 [2 – 1050]
<u>Clone number:-</u>	DU 30195
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	Glutathione Sepharose

Calculated molecular mass:-

Monoisotopic 139, 355.14 daltons
Average Mass 139, 423.48 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.61

Purity:- 60 %

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 buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-

KKAGAGSGQLIDSMANSFVGTR Final concentration: 300 µM

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Clone Data Sheet

ULK1 [2 – 1050]

Protein ULK1 [2 - 1050]

Clone number DU 30195

Species Human

Accession number NM_003565

Tags N-terminal GST

**Baculovirus
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKK
FELGLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERA
EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLP EMLKMFED
RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVL
QGPLGSLEPGRGGTETVGF~~EF~~SRKDLIGHGAFVVF~~KGRHREKH~~
DLEVAVKCIKKNLAKSQ~~TLLGKEIKILKELKHENIVALYDFQEM~~
ANSVYLVMEYCNGGDLADYLHAMRTLSEDTIRLFLOQIAGAMRLL
HSKGI IHRDLKPQNILLSNPAGRRANPNSIRVKIADFGFARYLQS
NMMAATLCGSPMYMAPEVIMSQHYDGKADLWSIGTIVYQCLTGKA
PFQASSPQDLRLFYEKNKTLVPTIPRETSAPLRQLLLALLQRNHK
DRMDFDEFFHHPFLDASPSVRKSPVPVPSYPSSGSGSSSSSSST
SHLASPPSLGEMQQLQKTLASPADTAGFLHSSRDSGGSKDSSCDT
DDFVMVPAQFPGDLVAEAPSAKPPPSLMSGSSLVASAGLESHG
RTPSPSPPCSSSPSPSGRAGPFSSSRCGASVPIPVPTQVQNYQRI
ERNLQSP~~TQFQ~~TPRSSAIRRSGSTSP~~LG~~FARASPPPAHAHGGV
LARKMSLGGRPYTPSPQVGTIPERPGWSGTPSPQGAEMRGRSP
RPGSSAPEHSPRTSGLGCR~~LHSAPNLSDLHVVRPKLPKPPTDPLG~~
AVFSPPQASPPQPSHGLQSCRNLRGSPKLPDFLQRNPLPPILGSP
TKAVPSFDFPKTPSSQNL~~LALLARQGVVMTPPRNRTL~~PD~~LSEVGP~~
FHGQPLG~~PGLRPGEDPKGPFGRSFSTSRLT~~DL~~LLKAAF~~GTQAPDL
GSTESLQEKPMIAPSAGFGGSLHPGARAGGTSSPSPVVFTV~~GSP~~
PSGSTPPQGP~~TRMFSAGPTGSASSARHLVPGPCSEAPAP~~ELPA
PGHGCSFADPIAANLEGAVTFEAPDLPEETLMEQE~~HT~~EILRGLRF
TLLFVQHVLEIAALKGSASEAAGGPEYQLQESV~~VADQISLLSREW~~
GFAEQLVLYLKVAELLSSGLQSAIDQIRAGKLC~~SSTVKQVVRRL~~
NELYKASVVSCQGLSLRLQRFFLDKQRLLDRIHSITAERLIFSHA
VQMVQSAALDEMFOHREGCVPRYHKALLLLEGLQHMLSDQADIEN
VTKCKLCIERLSALLTGICA

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Native sequence Amino acids E2 – A1050 (end) of human ULK1.
Residue E233 of the fusion protein is equivalent to E2 of the native enzyme. The GST tag is located at residues 1 - 220.

The following amino acid substitution is present
P – L, where P714 of the native enzyme is L945 of the fusion protein

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Bam*H1 and *Not*I sites of pFB-GST

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**Nucleotide
sequence of insert**

ggatccctgGAGCCCGGCCGCGGCGGCACAGAGACCGTGGGCAAG
TTCGAGTTC'TCCCGCAAGGACCTGATCGGCCACGGCGCCTTCGCG
GTGGTCTTCAAGGGCCGCCACCGCGAGAAGCACGATTTGGAGGTC
GCCGTCAAGTGCATTAACAAGAAGAACC'TCGCCAAGTCTCAGACG
CTGCTGGGGAAGGAAATCAAAATCCTGAAGGAACTGAAACATGAA
AACATCGTGGCCCTGTACGACTTCCAGGAAATGGCTAAT'TCTGTC
TACCTGGTTATGGAGTACTGCAACGGTGGGGACCTGGCCGACTAC
CTGCACGCCATGCGCACGCTGAGCGAGGACACCATCAGGCTCTTC
CTGCAGCAGATCGCGGGCGCCATGCGGCTTCTGCACAGCAAAGGC
ATCATCCACCGCGACCTGAAACCGCAGAACATCCTGCTGTCCAAC
CCCGCCGGCCGCGCGCCAACCCCAACAGCATCCGCGTCAAGATC
GCTGACTTCGGCTTCGCGCGGTACCTCCAGAGCAACATGATGGCG
GCCACACTCTGCGGCTCCCCATGTACATGGCCCCCGAGGTCATC
ATGTCCCAGCACTACGACGGGAAGGCGGACCTGTGGAGCATCGGC
ACCATCGTCTACCAGTGCCTGACGGGGAAGGCGCCCTTCCAGGCC
AGCAGCCCCAGGACCTGCGCCTGTTCTACGAGAAGAACAAGACG
TTGGTCCCCACCATCCCCGGGAGACCTCGGCCCGCTGCGGCAG
CTGCTCCTGGCCCTACTGCAACGCAACCACAAGGACCCGCATGGAC
TTCGATGAGTTTTTTTCATCACCTTTTCTCGATGCCAGCCCCTCG
GTCAGGAAATCCCCACCCGTGCCTGTGCCCTCGTACCCAAGCTCG
GGGTCCGGCAGCAGCTCCAGCAGCAGCTCCACCTCCCACCTGGCC
TCCCCGCGTCCCTGGGCGAGATGCAGCAGCTGCAGAAGACCCTG
GCCTCCCCGGCTGACACCGCTGGCTTCTTGCACAGCTCCCGGGAC
TCTGGTGGCAGCAAGGACTCTTCTGTGACACAGATGACTTTCGTC
ATGGTCCCCGCGCAGTTTCCAGGTGACCTGGTGGCTGAGGCGCCC
AGTGCCAAACCCCGCCAGACAGCCTGATGTGCAGTGGGAGCTCA
CTGGTGGCCTCTGCGGGCTTGGAGAGCCACGGCCGGACCCCATCT
CCATCCCCACCCTGCAGCAGCTCCCCCAGTCCCTCAGGCCGGGCT
GGCCCGTTCTCCAGCAGCAGGTGCGGCGCCTCTGTCCCATCCCA
GTCCCCACGCAGGTGCAGAACTACCAGCGCATTGAGCGAAACCTG
CAGTACCCACCCAGTTCCAAACACCTCGGTCTCTGCCATCCGC
AGGTCAGGCAGCACCAGCCCCCTGGGCTTTGCAAGGGCCAGCCCC
TCGCCCCCTGCCACGCTGAGCATGGAGGCGTCTGGCCAGGAAG
ATGTCTCTGGGTGGAGGCCGGCCCTACACGCCATCTCCTCAAGTT
GGAACCATCCCTGAGCGGCCAGGCTGGAGCGGGACGCCCTCCCCA
CAGGGAGCTGAGATGCGGGGTGGCAGGTCCCTCGTCCAGGCTCC
TCTGCACCCGAGCACTCTCCCCGCACTTCCGGGCTGGGCTGCCGC
CTGCACAGCGCCCCAACCTGTCTGACTTGCACGTCGTCCGCCCC
AAGCTGCCCAAACCCCCACGGACCCCTGGGAGCTGTGTTTCCAGC
CCACCACAGGCCAGCCCTCCCCAGCCGTCCACGGCCTGCAGTCC
TGCCGGAACCTGCGGGGCTCACCCAAGCTGCCCGACTTCTGCAG
CGAAACCCCTGCCCCCCATCCTGGGCTCCCCACCAAGGCTGTG

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CCCTCCTTTGACTTCCCGAAGACCCCCAGCTCCCAGAACCTGCTG
GCCCTCCTAGCCCGGCAGGGCGTGGTGATGACGCCCCCTCGAAAC
CGGACGCTGCCCCGACCTCTCGGAGGTGGGACCCTTCCATGGTCAG
CCGTTGGGCCCTGGCCTGCGGCCAGGCGAGGACCCCAAGGGCCCC
TTTGGCCGGTCTTTCAGCACCAGCCGCCTCACTGACCTGCTCCTT
AAGGCGGCGTTTGGGACACAAGCCCCGGACCTGGGCAGCACGGAG
AGCCTGCAGGAGAAGCCCATGGAGATCGCACCCCTCAGCTGGCTTT
GGAGGGAGCCTGCACCCAGGAGCCCGTGCTGGGGGCACCAGCAGC
CCTTCCCCGGTGGTCTTCACCGTGGGCTCTCCCCGAGCGGGAGC
ACGCCCCCCCAGGGCCCCCGCACCAGGATGTTCTCAGCGGGCCCC
ACTGGCTCTGCCAGCTCTTCTGCCCGCCACCTGGTGCCCTGGGCC
TGCAGCGAGGCCCCAGCCCCTGAGCTCCCTGCTCCAGGACACGGC
TGCAGCTTTGCCGACCCCATTTGCTGCGAACCTGGAGGGGGCTGTG
ACCTTCGAGGCCCCCGACCTCCCTGAGGAGACCCTCATGGAGCAA
GAGCACACGGAGATCCTGCGTGGCCTGCGCTTCACGCTGCTGTTT
GTGCAGCACGTCTTGGAGATCGCAGCCCTGAAGGGCAGCGCCAGT
GAGGCGGCGGGGGGCCCTGAGTACCAGCTGCAGGAGAGTGTGGTG
GCCGACCAGATCAGCCTGCTGAGCCGAGAATGGGGCTTCGCGGAA
CAGCTGGTGCTGTACCTGAAGGTGGCCGAGCTACTGTCTCCGGC
CTGCAAAGTGCCATCGACCAGATCCGGGCCGGCAAGCTCTGCCTG
TCGTCCACTGTGAAGCAGGTGGTGCGCAGGCTGAATGAGCTGTAC
AAGGCCAGCGTGGTGTCTTCCAGGGCCTGAGCCTGCGGCTGCAG
CGCTTCTTCCCTGGACAAGCAGCGGCTCCTGGACCGCATTCACAGC
ATCACTGCCGAGAGGCTCATCTTCAGCCACGCTGTGCAGATGGTG
CAGTCGGCTGCCCTGGACGAGATGTTCCAGCACCGTGAGGGCTGC
GTCCCACGCTACCACAAGGCCCTGCTGCTCCTGGAGGGGGCTGCAG
CACATGCTCTCGGACCAGGCCGACATCGAGAACGTCACCAAGTGC
AAGCTGTGCATTGAGCGGAGACTCTCGGCGCTGCTGACTGGCATC
TGTGCCtgagcggccgc