

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of active ULK2 [1 – 1036]**

<b><u>Enzyme description:-</u></b>	ULK2 [1 – 1036]
<b><u>Clone number:-</u></b>	DU 30989
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system
<b><u>Tag:-</u></b>	N-terminal GST
<b><u>Purification method:-</u></b>	Glutathione Sepharose

**Calculated molecular mass:-**

Monoisotopic      140, 439.60 daltons  
Average Mass      140, 529.53 daltons  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-**                      8.57

**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**

**ULK2 [1 – 1036]**

**Protein** ULK2 [1 - 1036]

**Clone number** DU 30989

**Species** Human

**Accession number** NM\_014683

**Tags** N-terminal GST

**Baculovirus  
expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK  
FELGLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERA  
EISMLEGAVLDIRYGVSRIAYSKDFETLKVDLFLSKLPEMLKMFED  
RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK  
KRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPKSDLEVL  
QGPLGSPGIPGSTRAAAMEVVGDFEYSKRDLVGHGAFVFRGRH  
RQKTDWEVAIKSINKKNLSKSQILLGKEIKILKELQHENIVALYD  
VQELPNSVFLVMEYCNGGDLADYLQAKGTLSEDTIRVFLHQIAAA  
MRILHSGKIIHRDLKPONILLSYANRRKSSVSGIRIKIADFGFAR  
YLHSNMMAATLCGSPMYMAPEVIMSQHYDAKADLWSIGTVIYQCL  
VGKPPFQANSPODLRMFYEKNRSLMPSIPRETSPYLANLLLGLLQ  
RNQKDRMDFEAFFSHPFLEQGPVKKSCPVPVPMYSGSVSGSSCGS  
SPSCRFASPPSLPDMQHIQEENLSSPPLGPPNYLQVSKDSASTSS  
KNSSCDTDDFVLVPHNISSDHSCDMPMGTAGRRASNEFLVCGGQC  
QPTVSPHSETAPIPVPTQIRNYQRIEQNLSTASSGTVNHGSPRS  
AVVRRSNTSPMGFLRPGSCSPVPADTAQTVGRRRLSTGSSRPYSPS  
PLVGTIPEQFSQCCCGHPQGHDSRSRNSSGSPVPOAQSPQSLLSG  
ARLQSAPTLTDIYQNKQKLRKHSDPVCPSHTGAGYSYSPQPSRP  
GSLGTSPTKHLGSSPRSSDWFVKTPPLPTIIGSPTKTTAPFKIPKT  
QASSNLLALVTRHGPAEEQSKDGNPRECAHCLLVQGSERQRAEQ  
QSKAVFGRSVSTGKLSDQOGKTPICRHQGSTDSLNTERPMDIAPA  
GACGGVLAPPAGTAASSKAVLFTVGSPPHSAAAPTCTHMFLRTRT  
TSVGPSNSGGSLCAMSGRVCVGSPPGPGFGSSPPGAEAAPSLRYV  
PYGASPPSLEGLITFEAPELPEETLMEREHTDTRLHNLNMLMTE  
CVLDLTAMRGGNPELCTSAVSLYQIQESVVVDQISQLSKDWGRVE  
QLVLYMKAQAQLLAASLHLAKAQIKSGKLSPSTAVKQVVKNLNERY  
KFCITMCKKLTEKLNRFSDKQRFIDEINSVTAEKLIYNCAVEMV  
QSAALDEMFOQTEDIVYRYHKAALLLEGLSRILQDPADIENVHKY  
KCSIERRLSALCHSTATV

**Native sequence** Amino acids M1 – V1036 (end) of human ULK2.  
Residue M243 of the fusion protein is equivalent to M1 of the  
native enzyme. The GST tag is located at residues 1 - 220.

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**Protease cleavage**      PreScission (LEVLFQGP) residues 221 - 228

**Cloning sites**            *NotI* sites of pFB-GST

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### **Nucleotide sequence of insert**

gcggccgcgATGGAGGTGGTGGGTGACTTCGAGTACAGCAAGAGG  
GATCTCGTGGGACACGGGGCCTTCGCCGTGGTCTTCCGGGGGCGG  
CACCGCCAGAAAAC TGATTGGGAGGTAGCTATTTAAAAGTATTAAT  
AAAAAGAACTTGTCAAAATCACAAATACTGCTTGGAAAGGAAATT  
AAAATCTTAAAGGAACTTCAGCATGAAAATATTGTAGCACTCTAT  
GATGTTT CAGGAATTACCCA ACTCTGTCTTTTTTGGTGATGGAGTAT  
TGCAATGGTGGAGACCTCGCAGATTATTTGCAAGCGAAAGGGACT  
CTCAGTGAAGACACGATCAGAGTGTCTTCTGCATCAGATTGCTGCT  
GCCATGCGAATCCTGCACAGCAAAGGAATCATCCACAGAGATCTC  
AAACCACAGAACATCTTGCTGTCTTATGCCAATCGCAGAAAATCA  
AGTGT CAGTGGTATTCGCATCAA AATAGCGGATTTTTGGTTTTGCT  
CGTTACCTACATAGTAACATGATGGCTGCAACACTGTGTGGATCC  
CCGATGTACATGGCTCCTGAGGTTATTATGTCTCAACATTATGAT  
GCTAAGGCTGACTTGTGGAGCATAGGAACAGTGATATAACCAATGC  
CTAGTTGGAAAACCACTTTTTCAGGCCAATAGTCTCAAGACTTA  
AGGATGTTTTATGAAAAAACAGGAGCTTAATGCCTAGTATTCCC  
AGAGAAACATCACCTTATTTGGCTAATCTCCTTTTGGGTTTGCTT  
CAGAGAAACCAAAAAGATAGAATGGACTTTGAAGCGTTTTTTTAGC  
CATCCTTTTCTTGAGCAAGGTCCAGTAAAAAATCTTGCCAGTT  
CCAGTGCCCATGTATTCTGGTTCTGTCTCTGGAAGCTCCTGTGGC  
AGCTCTCCATCTTGTCTGTTTTGCTTCTCCACCATCCCTTCCAGAT  
ATGCAGCATATTCAGGAAGAAAAC TTATCTTCCCCACCATTGGGT  
CCTCCCAACTATCTACAAGTTTTCAAAGATTCTGCCAGTACTAGT  
AGCAAGAACTCTTCTTGTGACACGGATGACTTTGTTTTGGTGCCA  
CACAACATCTCGTCAGACCACTCATGTGATATGCCAATGGGGACT  
GCTGGCAGACGTGCTTCAAATGAATTCTTGGTGTGTGGAGGGCAG  
TGTCAGCCTACTGTGTCACCTCACAGCGAAACAGCACCAATTCCA  
GTTCC TACTCAAATAAGGAATTATCAGCGCATAGAGCAGAATCTT  
ACATCTACTGCCAGCTCAGGCACAAATGTACATGGTTCTCCAAGA  
TCTGCAGTGGTACGAAGGTCCAACACCAGCCCCATGGGCTTCCCTC  
CGGCCGGGATCATGCTCCCCAGTACCAGCAGACACAGCACAGACA  
GTTGGACGAAGGCTCTCCACTGGGTCTTCTAGGCCTTACTCACCT  
TCCCCTTTGGTTGGTACCATTCTGAGCAATTCAGTCAGTGCTGC  
TGTGGGCATCCTCAGGGCCATGACTCCAGGAGTAGAAAAC TCTCA  
GGTTCTCCAGTGCCACAAGCTCAGTCCCACAGTCTCTCTTATCG  
GGTGCTAGACTGCAGAGCGCCCCACCCTCACTGACATCTATCAG  
AACAAGCAGAAGCTCAGAAAACAGCACTCTGACCCCGTGTGCCA  
TCCCATACTGGGGCTGGGTACAGCTACTCGCCTCAGCCCAGTCGG  
CCTGGCAGCCTTGGAACTTCTCCCACCAAGCACTTGGGGTCTCT  
CCACGGAGTTCTGACTGGTTCTTTAAAAC TCTTTGCCAACAATC  
ATTGGCTCTCCTACTAAGACCACAGCTCCTTTCAA AATCCCTAAA  
ACTCAAGCATCTTCCAACCTGTTAGCCTTGGTTACTCGTCATGGG

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CCTGCTGAAGAACAGTCGAAAGATGGGAATGAGCCACGGGAATGT  
GCCCATTCCTCTTAGTGCAAGGAAGTGAGAGGCAGCGGGCCGAG  
CAGCAGAGCAAGGCAGTGTTTGGCAGATCTGTCAGTACCGGGAAG  
TTATCAGATCAACAAGGAAAGACTCCTATATGTCGACATCAGGGC  
AGCACAGACAGTTTAAATACAGAACGACCAATGGATATAGCTCCG  
GCAGGAGCCTGTGGTGGTGTCTTGGCACCTCCTGCAGGTACAGCA  
GCAAGTTCCAAGGCTGTCTCTTCACTGTAGGGTCTCCTCCACAC  
AGTGCGGCAGCCCCACTTGTACCCACATGTTCTTTCGAACAAGA  
ACAACCTCAGTGGGGCCAGCAACTCCGGGGGCTCTCTTTGTGCC  
ATGAGTGGCCGCGTGTGCGTGGGGTCCCCGCCTGGCCCAGGCTTC  
GGCTCTTCCCCTCCAGGAGCAGAGGCAGCTCCAGCCTGAGATAC  
GTGCCTTACGGTGCTTACCCCCAGCCTAGAGGGGCTCATCACC  
TTTGAAGCCCCTGAACTGCCGGAGGAGACGCTGATGGAGCGGGAA  
CACACAGACACCTTACGCCATCTGAATGTGATGCTGATGTTCACT  
GAGTGTGTGCTGGACCTGACAGCCATGAGGGGAGGAAACCCTGAG  
CTGTGCACATCTGCTGTGTCCTTGTACCAGATCCAGGAGAGTGTG  
GTGGTGGACCAAATCAGTCAGCTGAGCAAAGACTGGGGGCGGGTG  
GAGCAGCTGGTGTGTACATGAAAGCAGCACAGCTGCTTGC GGCT  
TCTCTGCATCTTGCCAAAGCCCAGATCAAGTCCGGGAAACTGAGT  
CCATCCACAGCTGTGAAACAAGTTGTCAAGAATCTGAACGAACGA  
TATAAATTCATGCATCACCATGTGCAAGAACTTACAGAAAAGCTG  
AATCGATTCTTCTCTGACAAACAGAGGTTTATTGATGAAATCAAC  
AGTGTGACTGCAGAGAAACTCATCTATAATTGTGCTGTAGAAATG  
GTTTCACTGCAGCCCTGGATGAGATGTTTCAGCAGACCGAAGAT  
ATTGTTTATCGCTATCATAAGGCAGCCCTTCTTTTGGAAAGCCTA  
AGTAGGATTCTACAGGACCCTGCAGATATTGAAAATGTGCATAAA  
TATAAATGTAGTATTGAGAGAAGACTGTGCGGCTCTGCCATAGC  
ACCGCAACCGTGtgagcgccgc