Certificate of Analysis

REACTION BIOLOGY

ProQinase™ ULK2

unc-51 like autophagy activating kinase 2

Recombinant Human Active Protein Kinase

HGNC Symbol: ULK2

Synonyms: ATG1B, KIAA0623, Unc51.2

Product No.: 1670-0000-1

Lot: 001

Description: Human ULK2, N-terminal fragment, amino acids M_1-P_{300} (as in NCBI/Protein entry NP_055498.3), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: ULK2 Lot 001, was confirmed as ULK2 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 61,853 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

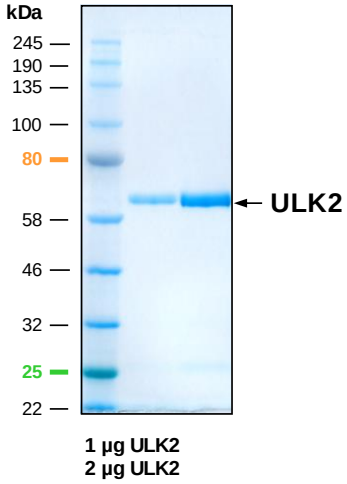
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

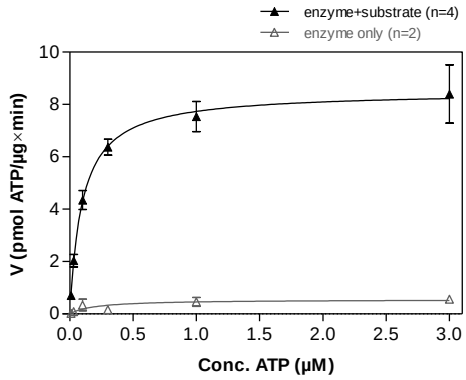
Protein concentration: 0.596 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 8.1 pmol/µg × min
ATP-K_M: 0.1 µM

ULK2 Lot 001:
Coomassie stain



ULK2 Lot 001:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: S6-derived peptide, 40 µg/ml
  Kinase: 0.4 µg/ml
- Filter binding assay
MSPH membrane (Millipore)

Recombinant Proteins

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ProQinase™ ULK2

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GST-ULK2 Recombinant Fusion Protein Amino Acid Sequence

```
   1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID   60
  61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV  120
 121  DFLSKLPEML KMFKDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK  180
 181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG  240
 241  PMEVVGDFEY SKRDLVGHGA FAVVFRGRHR QKTDWEVAIK SINKKNLSKS QILLGKEIKI  300
 301  LKELQHENIV ALYDVQELPN SVFLVMEYCN GGDLADYLQA KGTLSEDTIR VFLHQIAAAM  360
 361  RILHSKGIIH RDLKPQNILL SYANRRKSSV SGIRIKIADF GFARYLHSNM MAATLCGSPM  420
 421  YMAPEVIMSQ HYDAKADLWS IGTVIYQCLV GKPPFQANSP QDLRMFYEKN RSLMPSIPRE  480
 481  TSPYLANLLL GLLQRNQKDR MDFEAFFSHP FLEQGPVKKS CPVPVPMYSG SVSGSSCGSS  540
 541  P                                                                  600
```

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: ULK2 fragment

ULK2 wt[1] Amino Acid Sequence

```
1     MEVVGDFEYS KRDLVGHGAF AVVFRGRHRQ KTDWEVAIKS INKKNLSKSQ ILLGKEIKIL   60
61    KELQHENIVA LYDVQELPNS VFLVMEYCNG GDLADYLQAK GTLSEDTIRV FLHQIAAAMR  120
121   ILHSKGIIHR DLKPQNILLS YANRRKSSVS GIRIKIADFG FARYLHSNMM AATLCGSPMY  180
181   MAPEVIMSQH YDAKADLWSI GTVIYQCLVG KPPFQANSPQ DLRMFYEKNR SLMPSIPRET  240
241   SPYLANLLLG LLQRNQKDRM DFEAFFSHPF LEQGPVKKSC PVPVPMYSGS VSGSSCGSSP  300
301   SCRFASPPSL PDMQHIQEEN LSSPPLGPPN YLQVSKDSAS TSSKNSSCDT DDFVLVPHNI  360
361   SSDHSCDMPV GTAGRRASNE FLVCGGQCQP TVSPHSETAP IPVPTQIRNY QRIEQNLTST  420
421   ASSGTNVHGS PRSAVVRRSN TSPMGFLRPG SCSPVPADTA QTVGRRLSTG SSRPYSPSPL  480
481   VGTIPEQFSQ CCCGHPQGHD SRSRNSSGSP VPQAQSPQSL LSGARLQSAP TLTDIYQNKQ  540
541   KLRKQHSDPV CPSHTGAGYS YSPQPSRPGS LGTSPTKHLG SSPRSSDWFF KTPLPTIIGS  600
601   PTKTTAPFKI PKTQASSNLL ALVTRHGPAE EQSKDGNEPR ECAHCLLVQG SERQRAEQQS  660
661   KAVFGRSVST GKLSDQQGKT PICRHQGSTD SLNTERPMDI APAGACGGVL APPAGTAASS  720
721   KAVLFTVGSP PHSAAAPTCT HMFLRTRTTS VGPSNSGGSL CAMSGRVCVG SPPGPGFGSS  780
781   PPGAEAAPSL RYVPYGASPP SLEGLITFEA PELPEETLME REHTDTLRHL NVMLMFTECV  840
841   LDLTAMRGGN PELCTSAVSL YQIQESVVVD QISQLSKDWG RVEQLVLYMK AAQLLAASLH  900
901   LAKAQIKSGK LSPSTAVKQV VKNLNERYKF CITMCKKLTE KLNRFFSDKQ RFIDEINSVT  960
961   AEKLIYNCAV EMVQSAALDE MFQQTEDIVY RYHKAALLLE GLSRILQDPA DIENVHKYKC  1020
1021  SIERRLSALC HSTATV                                                 1080
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blue: ULK2 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_055498.3

Recombinant Proteins

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