Certificate of Analysis

REACTION BIOLOGY

ProQinase™ ULK3

unc-51 like kinase 3

Recombinant Human Active Protein Kinase

HGNC Symbol: ULK3

Synonyms: DKFZP434C131; FLJ90566

Product No.: 1939-0000-1

Lot: 002

Description: Human ULK3, full length, amino acids M_1-Q_{472} (as in NCBI/Protein entry NP_001092906.3), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: ULK3 Lot 002, was confirmed as ULK3 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 81534 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

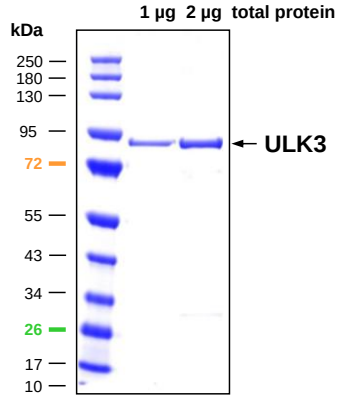
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

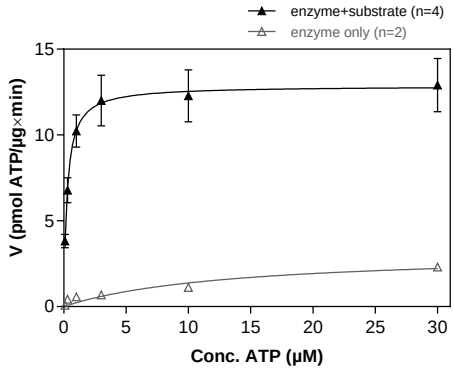
Protein concentration: 0.484 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 13 pmol/µg*min
ATP-K_M: 0.25 µM

ULK3 Lot 002:
Coomassie stain



ULK3 Lot 002:
Determination of V_{max} and K_M value for ATP



- 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: Casein 100 µg/ml
  Kinase: 1 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Recombinant Proteins

Sequence information

GST-ULK3 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  PMAGPGWGPP RLDGFILTER LGSGTYATVY KAYAKKDTRE VVAIKCVAKK SLNKASVENL  300
 301  LTEIEILKGI RHPHIVQLKD FQWDSDNIYL IMEFCAGGDL SRFIHTRRIL PEKVARVFMQ  360
 361  QLASALQFLH ERNISHLDLK PQNILLSSLE KPHLKLADFG FAQHMSPWDE KHVLRGSPLY  420
 421  MAPEMVCQRQ YDARVDLWSM GVILYEALFG QPPFASRSFS ELEEKIRSNR VIELPLRPLL  480
 481  SRDCRDLLQR LLERDPSRRI SFQDFFAHPW VDLEHMPSGE SLGRATALVV QAVKKDQEGD  540
 541  SAAALSLYCK ALDFFVPALH YEVDAQRKEA IKAKVGQYVS RAEELKAIVS SSNQALLRQG  600
 601  TSARDLLREM ARDKPRLLAA LEVASAAMAK EEAAGGEQDA LDLYQHSLGE LLLLLAAEPP  660
 661  GRRRELLHTE VQNLMARAEY LKEQVKMRES RWEADTLDKE GLSESVRSSC TLQ         720
```

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: ULK3

ULK wt[1] Amino Acid Sequence

```
1    MAGPGWGPPR LDGFILTERL GSGTYATVYK AYAKKDTREV VAIKCVAKKS LNKASVENLL   60
61   TEIEILKGIR HPHIVQLKDF QWDSDNIYLI MEFCAGGDLS RFIHTRRILP EKVARVFMQQ  120
121  LASALQFLHE RNISHLDLKP QNILLSSLEK PHLKLADFGF AQHMSPWDEK HVLRGSPLYM  180
181  APEMVCQRQY DARVDLWSMG VILYEALFGQ PPFASRSFSE LEEKIRSNRV IELPLRPLLS  240
241  RDCRDLLQRL LERDPSRRIS FQDFFAHPWV DLEHMPSGES LGRATALVVQ AVKKDQEGDS  300
301  AAALSLYCKA LDFFVPALHY EVDAQRKEAI KAKVGQYVSR AEELKAIVSS SNQALLRQGT  360
361  SARDLLREMA RDKPRLLAAL EVASAAMAKE EAAGGEQDAL DLYQHSLGEL LLLLAAEPPG  420
421  RRRELLHTEV QNLMARAEYL KEQVKMRESR WEADTLDKEG LSESVRSSCT LQ          480
```

blue: ULK3 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_001092906.3

Recombinant Proteins