Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ AKT3 aa1-479

AKT serine/threonine kinase 3

Recombinant Human Active Protein Kinase

HGNC Symbol: AKT3

Synonyms: PKBG, PKB gamma, PRKBG, RAC-gamma, RAC-PK-gamma, STK-2, DKFZp434N0250

Product No.: 1578-0000-1

Lot: 003

Description: Human Kinase, full length, amino acids M_1-E_{479} (as in NCBI/Protein entry NP_005456.1), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: AKT3 aa1-479 Lot 003, was confirmed as AKT3 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 83,838 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: With PDK1

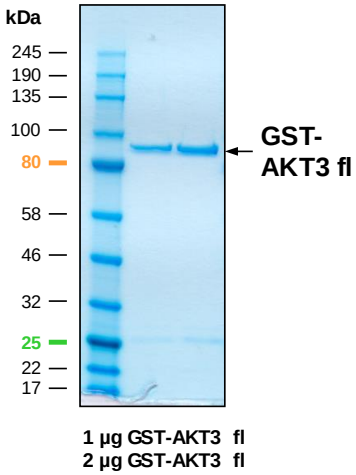
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

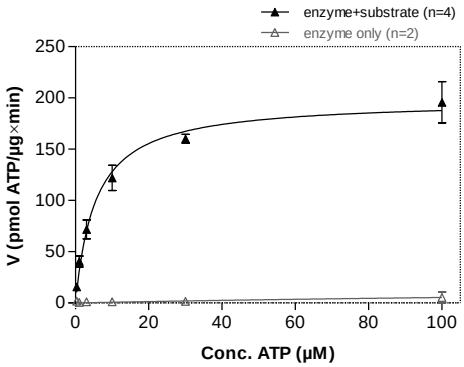
Protein concentration: 0.491 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 198 pmol/µg × min
ATP-K_M: 5.5 µM

AKT3 aa1-479 Lot 003: Coomassie stain



AKT3 aa1-479 Lot 003: 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: GSK3-derived peptide 100 µg/ml
  Kinase: 1 µg/ml
- Filter binding assay

MSPH membrane (Millipore)

Additional assay technology:
AKT3 aa1-479 Lot 003 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

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GST-AKT1 aa1-479 Recombinant Fusion Protein Amino Acid Sequence

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  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 PMSDVTIVKE GWVQKRGEYI KNWRPRYFLL KTDGSFIGYK EKPQDVDLPY PLNNFSVAKC 300
301 QLMKTERPKP NTFIIRCLQW TTVIERTFHV DTPEEREEWT EAIQAVADRL QRQEEERMNC 360
361 SPTSQIDNIG EEEMDASTTH HKRKTMNDFD YLKLLGKGTF GKVILVREKA SGKYYAMKIL 420
421 KKEVIIAKDE VAHTLTESRV LKNTRHPFLT SLKYSFQTKD RLCFVMEYVN GGELFFHSSR 480
481 ERVFSEDRTR FYGAEIVSAL DYLHSGKIVY RDLKLENLML DKDGHIKITD FGLCKEGITD 540
541 AATMKTFCGT PEYLAPEVLE DNDYGRAVDW WGLGVVMYEM MCGRLPFYNQ DHEKLFELIL 600
601 MEDIKFPRTL SSDAKSLLSG LLIKDPNKRL GGGPDDAKEI MRHSFFSGVN WQDVYDKKLV 660
661 PPFKPQVTSE TDTRYFDEEF TAQTITITPP EKYDEDGMDC MDNERRPHFP QFSYSASGRE 720
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1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: AKT3

AKT3 wt[1] Amino Acid Sequence

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  1 MSDVTIVKEG WVQKRGEYIK NWRPRYFLLK TDGSFIGYKE KPQDVDLPYP LNNFSVAKCQ  60
 61 LMKTERPKPN TFIIRCLQWT TVIERTFHVD TPEEREEWTE AIQAVADRLQ RQEEERMNCS 120
121 PTSQIDNIGE EEMDASTTHH KRKTMNDFDY LKLLGKGTFG KVILVREKAS GKYYAMKILK 180
181 KEVIIAKDEV AHTLTESRVL KNTRHPFLTS LKYSFQTKDR LCFVMEYVNG GELFFHLSRE 240
241 RVFSEDRTRF YGAEIVSALD YLHSGKIVYR DLKLENLMLD KDGHIKITDF GLCKEGITDA 300
301 ATMKTFCGTP EYLAPEVLED NDYGRAVDWW GLGVVMYEMM CGRLPFYNQD HEKLFELILM 360
361 EDIKFPRTLS SDAKSLLSGL LIKDPNKRLG GGPDDAKEIM RHSFFSGVNW QDVYDKKLVP 420
421 PFKPQVTSET DTRYFDEEFT AQTITITPPE KYDEDGMDCM DNERRPHFPQ FSYSASGRE  480
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blue: AKT3 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_005456.1

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