Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ AKT1 aa106-480

AKT serine/threonine kinase 1

Recombinant Human Active Protein Kinase

HGNC Symbol: AKT1

Synonyms: PKB, RAC, PRKBA, RAC-ALPHA

Product No.: 0132-0000-2

Lot: 007

Description: Human AKT1, C-terminal fragment, amino acids V_{106}-A_{480} (as in NCBI/Protein entry NP_005154.1), N-terminal GST-HIS_6, C-terminal HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: AKT1 Lot 007, was confirmed as AKT1 by Western Blotting using a specific anti AKT1 antibody

Theoretical $MW_{Fusion\ Protein}$: 77,832 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: With PDK1

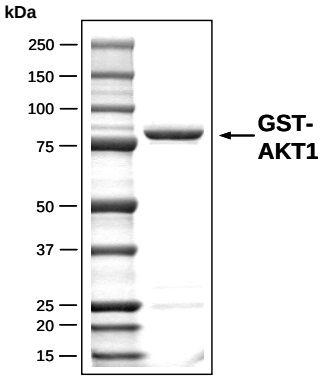
Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

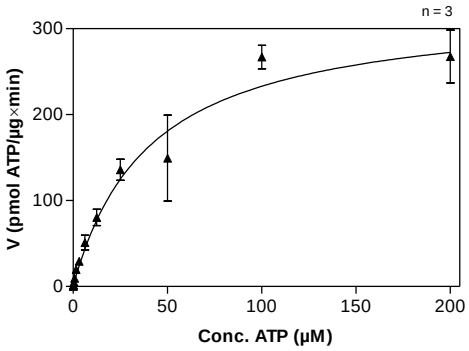
Biochemical Parameters:
Specific kinase activity (P_i transfer): 328 pmol/µg × min
ATP-K_M: 41 µM

AKT1 aa106-480 Lot 007: Coomassie stain



2.0 µg GST-AKT1

AKT1 aa106-480 Lot 007: 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: R_{11}-GSK3(14-27), 100 µg/ml
  AKT1: 2 µg/ml
- Filter binding assay

MSPH membrane (Millipore)

Additional assay technology:
AKT1 aa106-480 Lot 007 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 aa106-480 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 KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI 240
241 LVPRGSPGLD GIYARGIQAS MGARGRLQGT PVADGLKKQE EEEMDFRSGS PSDNSGAEEM 300
301 EVSLAKPKHR VTMNEFEYLK LLGKGTFGKV ILVKEKATGR YYAMKILKKE VIVAKDEVAH 360
361 TLTENRVLQN SRHPFLTALK YSFQTHDRLC FVMEYANGGE LFFHLSRERV FSEDRARFYG 420
421 AEIVSALDYL HSEKNVVYRD LKLENLMLDK DGHIKITDFG LCKEGIKDGA TMKTFCGTPE 480
481 YLAPEVLEDN DYGRAVDWWG LGVVMYEMMC GRLPFYNQDH EKLFELILME EIRFPRTLGP 540
541 EAKSLLSGLL KKDPKQRLGG GSEDAKEIMQ HRFFAGIVWQ HVYEKKLSPP FKPQVTSETD 600
601 TRYFDEEFTA QMITITPPDQ DDSMECVDSE RRPHFPQFSY SASSTAKGEL RGHPFEGKPI 660
661 PNPLLGLDST RTGHHHHHH                                              720
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1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: AKT1 fragment

AKT1 wt[1] Amino Acid Sequence

```
  1 MSDVAIVKEG WLHKRGEYIK TWRPRYFLLK NDGTFIGYKE RPQDVDQREA PLNNFSVAQC  60
 61 QLMKTERPRP NTFIIRCLQW TTVIERTFHV ETPEEREEWT TAIQTVADGL KKQEEEEMDF 120
121 RSGSPSDNSG AEEMEVSLAK PKHRVTMNEF EYLKLLGKGT FGKVILVKEK ATGRYYAMKI 180
181 LKKEVIVAKD EVAHTLTENR VLQNSRHPFL TALKYSFQTH DRLCFVMEYA NGGELFFHLS 240
241 RERVFSEDRA RFYGAEIVSA LDYLHSEKNV VYRDLKLENL MLDKDGHIKI TDFGLCKEGI 300
301 KDGATMKTFC GTPEYLAPEV LEDNDYGRAV DWWGLGVVMY EMMCGRLPFY NQDHEKLFEL 360
361 ILMEEIRFPR TLGPEAKSLL SGLLKKDPKQ RLGGGSEDAK EIMQHRFFAG IVWQHVYEKK 420
421 LSPPFKPQVT SETDTRYFDE EFTAQMITIT PPDQDDSMEC VDSERRPHFP QFSYSASSTA 480
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blue: AKT1 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_005154.1

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