Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ AKT1 aa1-480

AKT serine/threonine kinase 1

Recombinant Human Active Protein Kinase

HGNC Symbol: AKT1

Synonyms: AKT, PKB, PRKBA, RAC, RAC-alpha

Product No.: 1379-0000-2

Lot: 004

Description: Human AKT1, full length, amino acids M_1-A_{480} (as in NCBI/Protein entry NP_005154.2), activated with PDK1, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: AKT1 wt Lot 004, was confirmed as AKT1 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 81,191 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: With PDK1

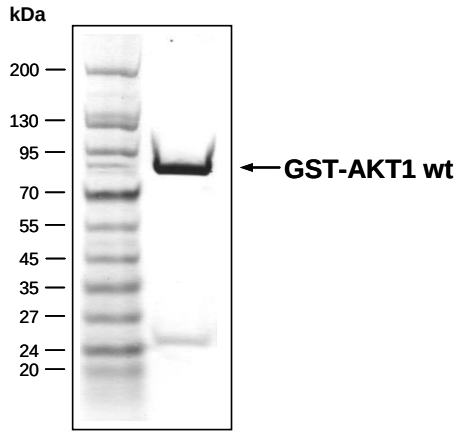
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

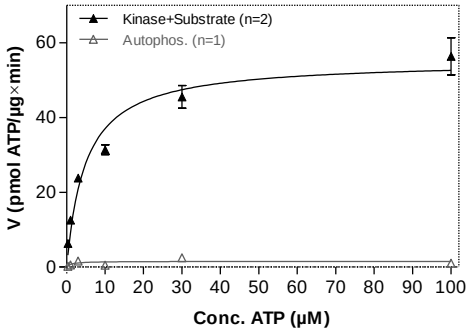
Biochemical Parameters:
Specific kinase activity (P_i transfer): 55 pmol/µg × min
ATP-K_M: 5 µM

AKT1 aa1-480 Lot 004:
Coomassie stain



2.0 µg GST-AKT1 wt

AKT1 aa1-480 Lot 004:
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 1-480: 2 µg/ml
- Filter binding assay
  MSPH membrane (Millipore)

Additional assay technology:
AKT1 aa1-480 Lot 004 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 aa1-480 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	PLAMVMSDVA IVKEGWLHKR GEYIKTWRPR YFLLKNDGTF IGYKERPQDV DQREAPLNNF	300
301	SVAQCQLMKT ERPRPNTFII RCLQWTTVIE RTFHVETPEE REEWTTAIQT VADGLKKQEE	360
361	EEMDFRSGSP SDNSGAEEME VSLAKPKHRV TMNEFEYLKL LGKGTFGKVI LVKEKATGRY	420
421	YAMKILKKEV IVAKDEVAHT LTENRVLQNS RHPFLTALKY SFQTHDRLCF VMEYANGGEL	480
481	FFHLSRERVF SEDRARFYGA EIVSALDYLH SEKNVVYRDL KLENLMLDKD GHIKITDFGL	540
541	CKEGIKDGAT MKTFCGTPEY LAPEVLEDND YGRAVDWWGL GVVMYEMMCG RLPFYNQDHE	600
601	KLFELILMEE IRFPRTLGPE AKSLLSGLLK KDPKQRLGGG SEDAKEIMQH RFFAGIVWQH	660
661	VYEKKLSPPF KPQVTSETDT RYFDEEFTAQ MITITPPDQD DSMECVDSER RPHFPQFSYS	720
721	ASGTA	780

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: AKT1

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 QFSYSASGTA	480

blue: AKT1 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_005154.2

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