

Certificate of Analysis



AKT3 aa106-479

v-akt murine thymoma viral oncogene homolog 2

Recombinant Human Active Protein Kinase

HGNC Symbol: AKT3

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

Product No.: 0436-0000-2

Lot: 004

Description: Human AKT3, C-terminal fragment, amino acids A₁₀₆-E₄₇₉ (as in NCBI/Protein entry NP_005456.1), activated, N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

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

Theoretical MW_{Fusion Protein}: 72,637 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: With PDK1

Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 4 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: 0.165 µg/µl

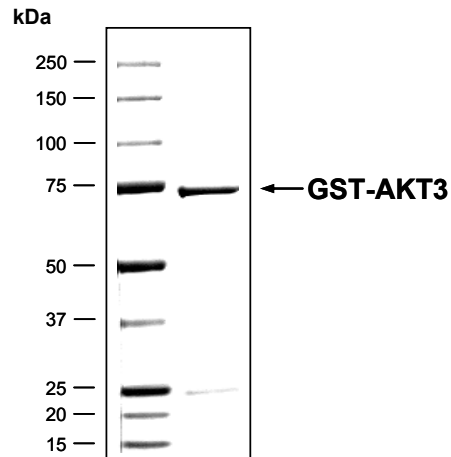
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 411 pmol/µg×min

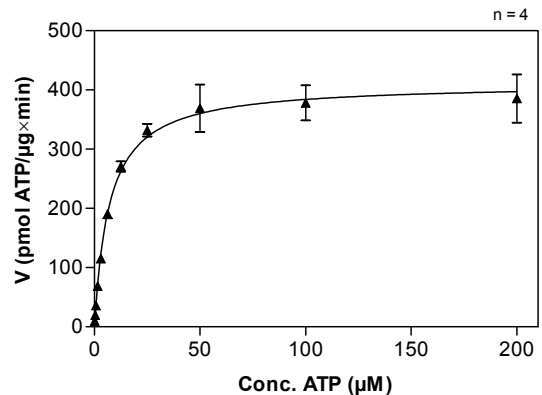
ATP-K_M: 7.1 µM

AKT3 Lot 004: Coomassie stain



2.0 µg GST-AKT3

AKT3 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 MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg / ml PEG_{20,000}
 - ATP (variable)
 - Substrate: GSK3(14-27), 100 µg / ml
 - AKT3: 4.0 µg / ml
- Filter binding assay
 - MSPH membrane (Millipore)

Additional assay technology: AKT3 Lot 004

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega



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AKT3 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGQWATF	GGGDHPPKSD	PMG HHHHHH HG	RRRASVAAGI	240
241	LVPRGS PGLD	GICSRADRLQ	RQEEERMNCS	PTSQIDNIGE	EEMDASTTHH	KRKTMNDFDY	300
301	LKLLGKGTFG	KVILVREKAS	GKYYAMKILK	KEVIIAKDEV	AHTLTESRVL	KNTRHPFLTS	360
361	LKYSFQTKDR	LCFVMEYVNG	GELFFH SRE	RVFSEDTRTF	YGAEIVSALD	YLHSGKIVYR	420
421	DLKLENMLD	KDGHKITDF	GLCKEGITDA	ATMKTFCGTP	EYLAPEVLED	NDYGRAVDWW	480
481	GLGVVYEMM	CGRLPFYNQD	HEKLFELILM	EDIKFPRTLS	SDAKSLLSGL	LIKDPNKRLG	540
541	GGPDDAKEIM	RHSFFSGVNW	QDVYDKKLV	PFKPQVTSET	DTRYFDEEFT	AQTITITPPE	600
601	KYDEDGMDCM	DNERRPHFPQ	FSYSASGRE				660

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: AKT3 fragment **boxed**: variation from RefSeq

AKT3 wt ¹ amino acid sequence							
1	MSDVTIVKEG	WVQKRGEYIK	NWRPRYFLK	TDGSFIGYKE	KPQDVLDPYP	LNNFSVAKCQ	60
61	LMKTERPKPN	TFIIRCLQWT	TVIERTFHVD	TPEEREWTE	AIQAVADRLQ	RQEEERMNCS	120
121	PTSQIDNIGE	EEMDASTTHH	KRKTMNDFDY	LKLLGKGTFG	KVILVREKAS	GKYYAMKILK	180
181	KEVIIAKDEV	AHTLTESRVL	KNTRHPFLTS	LKYSFQTKDR	LCFVMEYVNG	GELFFH SRE	240
241	RVFSEDTRTF	YGAEIVSALD	YLHSGKIVYR	DLKLENMLD	KDGHKITDF	GLCKEGITDA	300
301	ATMKTFCGTP	EYLAPEVLED	NDYGRAVDWW	GLGVVYEMM	CGRLPFYNQD	HEKLFELILM	360
361	EDIKFPRTLS	SDAKSLLSGL	LIKDPNKRLG	GGPDDAKEIM	RHSFFSGVNW	QDVYDKKLV	420
421	PFKPQVTSET	DTRYFDEEFT	AQTITITPPE	KYDEDGMDCM	DNERRPHFPQ	FSYSASGRE	480

blue: AKT3 sequence expressed in fusionprotein **Red**: variant in fusionprotein

¹NCBI/Protein accession number NP_005456.1