KinaseDetect ApS
http://www.kinasedetect.com

Technical support:
Phone: +45 66 10 66 62
e-mail:support@kinasedetect.com

Certificate of Analysis

Product	**AKT3/PKBgamma, active** **human recombinant, expressed in Sf9 cells, N-His-fusion protein**
Cat No	PK-004-01
Lot No	151204
Description	Purified recombinant human AKT3 kinase (PKBgamma) expressed in Sf9 cells. Active form of AKT3 kinase. Phosphorylated at Thr305 and Ser472. Suitable for labeling AKT3 kinase substrates. Features a polyhistidine tag to facilitate removal of AKT3 kinase from the reaction mixture. Purified by Ni-NTA agarose chromatography. Sequence based calculated M.W. 60,508 Approved HUGO gene symbol: AKT3 Synonyms: v-akt murine thymoma viral oncogene homolog 3, protein kinase B gamma, PKBG, RAC-gamma, PRKBG

Quality		
	Protein concentration (Bradford with BSA as standard)	1 mg/ml
	Purity	> 95% by SDS PAGE
	Specific activity (* 1 Unit is defined as 1 picomole phosphate transferred to synthetic peptide (RPRAATF) per min at 30 °C)	467,100 Units*/ mg
	Protease activity (Twining test)	none

Form	Liquid. In 50 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, 50 % glycerol, pH 8.5.
Package size	20 microgram
Storage condition	-70 ^{O}C
Shipment conditions	dry ice

Material for in vitro research use only. Not for pharmaceutical or drug application. Material does not contain any animal products such as albumin.

AVOID FREEZE/THAW CYCLES

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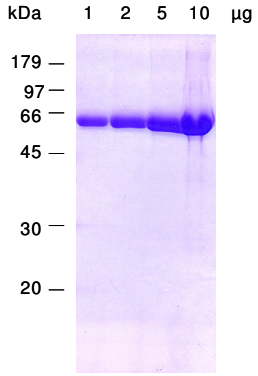
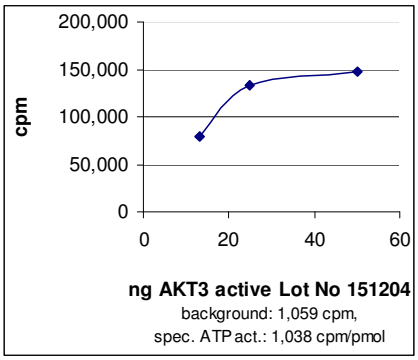
SDS-PAGE analysis



Activity determination



Amino acid sequence information

M32 corresponds to M1 in AF135794, E510 corresponds to the last amino acid in AF135794

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MSYYHHHHHH DYDIPTTENL YFQGAMGIRN SMSDVTIVKE GWVQKRGEYI  50
KNWRPRYFLL KTDGSFIGYK EKPQDVDLPY PLNNFSVAKC QLMKTERPKP 100
NTFIIRCLQW TTVIERTFHV DTPEEREEWT EAIQAVADRL QRQEEERMNC 150
SPTSQIDNIG EEEMDASTTH HKRKTMNDFD YLKLLGKGTF GKVILVREKA 200
SGKYYAMKIL KKEVIIAKDE VAHTLTESRV LKNTRHPFLT SLKYSFQTKD 250
RLCFVMEYVN GGELFFHLSR ERVFSEDRTR FYGAEIVSAL DYLHSGKIVY 300
RDLKLENLML DKDGHIKITD FGLCKEGITD AATMKTFCGT PEYLAPEVLE 350
DNDYGRAVDW WGLGVVMYEM MCGRLPFYNQ DHEKLFELIL MEDIKFPRTL 400
SSDAKSLLSG LLIKDPNKRL GGGPDDAKEI MRHSFFSGVN WQDVYDKKLV 450
PPFKPQVTSE TDTRYFDEEF TAQTITITPP EKYDEDGMDY MDNERRPHFP 500
QFSYSASGRE GTKLVEKY                                   518
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In vitro Kinase Assay

Assay components

Enzyme dilution buffer (EDB): 50 mM Tris pH 7.5, 0.1 mM EGTA, 10 mM DTT, 1 mg/ml BSA

One-For-All-Buffer (OFAB): 20 mM Tris-HCl, 25 mM beta-glycerol phosphate, 5 mM EGTA, 1 mM sodium orthovanadate, 1 mM DTT, pH 7.5

Substrate: AKT peptide, RPRAATF, 400 µM

Protein kinase: AKT3, 5-50 ng/microliter diluted in EDB

Magnesium/ATP Cocktail: 75 mM $MgCl_2$, 500 microM ATP

Diluted [gamma–^{32}P]ATP: Mix 197 microliter Magnesium/ATP cocktail with 3 microliter (30 microCi) [gamma–^{32}P]ATP (3,000 Ci/mmol, e.g. from Hartmann Analytic, Braunschweig, Germany)

Assay procedure

All compounds are pipetted into a microcentrifuge tube on ice

1. Add 10 microliter OFAB
2. Add 10 microliter 400 microM AKT peptide
3. Add 10 microliter AKT3 enzyme (50 - 500 nanogram/assay)
4. Add 10 microliter of the diluted [gamma–^{32}P]ATP
5. Incubate 10 min at 30 °C.
6. Stop the reaction by setting samples on ice
7. Remove 10 microliter and spot on P81 paper (let bind to the paper for 30 sec)
8. Immerse the paper in 0.75% phosphoric acid, gently shake on a rotator
9. Wash 3 x with phosphoric acid
10. Wash 1 x with acetone
11. Dry under infrared light
12. Read in scintillation counter or Instant Imager