KinaseDetect ApS
http://www.kinasedetect.com

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

Certificate of Analysis

Product	**AKT2/PKBbeta, active** **human recombinant, expressed in Sf9 cells, N-His-fusion protein**
Cat No	PK-003-01
Lot No	070704
Description	Purified recombinant human AKT2 kinase (PKBbeta) expressed in Sf9 cells. Active form of AKT2 kinase. Phosphorylated at Thr309 and Ser474. Suitable for labeling AKT2 kinase substrates. Features a polyhistidine tag to facilitate removal of AKT2 kinase from the reaction mixture. Purified by Ni-NTA agarose chromatography. Sequence based calculated M.W. 60,442 Approved HUGO gene symbol: AKT2 Synonyms: v-akt murine thymoma viral oncogene homolog 2, protein kinase B beta

Quality		
	Protein concentration (Bradford with BSA as standard)	1.54 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	6,100 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	100 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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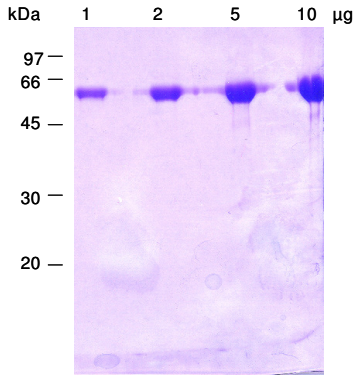
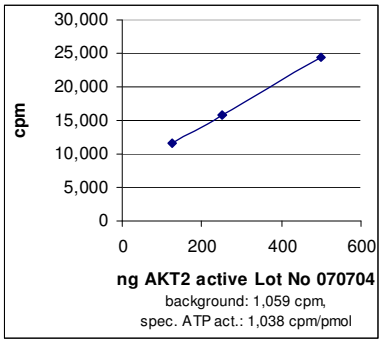
SDS-PAGE analysis



Activity determination



Amino acid sequence information

M32 corresponds to M1 in NM_001626, E512 is the last amino acid in NM_001626

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MSYYHHHHHH DYDIPTTENL YFQGAMGIRN SMNEVSVIKE GWLHKRGEYI  50
KTWRPRYFLL KSDGSFIGYK ERPEAPDQTL PPLNNFSVAE CQLMKTERPR 100
PNTFVIRCLQ WTTVIERTFH VDSPDEREEW MRAIQMVANS LKQRAPGEDP 150
MDYKCGSPSD SSTTEEMEVA VSKARAKVTM NDFDYLKLLG KGTFGKVILV 200
REKATGRYYA MKILRKEVII AKDEVAHTVT ESRVLQNTRH PFLTALKYAF 250
QTHDRLCFVM EYANGGELFF HLSRERVFTE ERARFYGAEI VSALEYLHSR 300
DVVYRDIKLE NLMLDKDGHI KITDFGLCKE GISDGATMKT FCGTPEYLAP 350
EVLEDNDYGR AVDWWGLGVV MYEMMCGRLP FYNQDHERLF ELILMEEIRF 400
PRTLSPEAKS LLAGLLKKDP KQRLGGGPSD AKEVMEHRFF LSINWQDVVQ 450
KKLLPPFKPQ VTSEVDTRYF DDEFTAQSIT ITPPDRYDSL GLLELDQRTH 500
FPQFSYSASI REGTKLVEKY                                  520
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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: AKT2, 100 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 AKT 2 enzyme (1 microgram/assay)
4. Add 10 microliter of the diluted [gamma–^{32}P]ATP
5. Incubate 10 min at 30 ºC.
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