KinaseDetect ApS
http://www.kinasedetect.com

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

Certificate of Analysis

Product	**Protein kinase C alpha (PRKCA), active human recombinant, expressed in Sf9 cells, C-His-fusion protein**
Cat No	PK-024-01
Lot No	260204
Description	Purified human recombinant protein kinase C alpha (PKCalpha), expressed in Sf9 cells. Active form. Suitable for labeling PKC substrates. Features a polyhistidine tag to facilitate removal of the enzyme from the reaction mixture. Sequence based calculated M.W. 81,912 Approved HUGO gene symbol: PRKCA Synonyms: PKCA, PKCalpha

Quality		
	Protein concentration (Bradford with BSA as standard)	0.4 mg/ml
	Purity	> 95 % by SDS PAGE
	Specific activity (* 1 Unit is defined as 1 picomole phosphate transferred to PKC peptide (QKRPSQRSKYL) per min at 30 °C)	355,000 Units*/ mg
	Protease activity (Twining test)	none

Form	Liquid. In 20 mM NaH_2PO4, 500 mM NaCl, 1 mM Na-orthovanadate, 5 mM NaF, 40 mM beta-glycerophosphat, 5 % glycerol, pH 7.8.
Package size	10 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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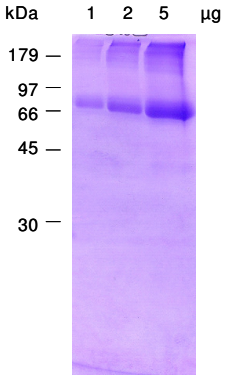
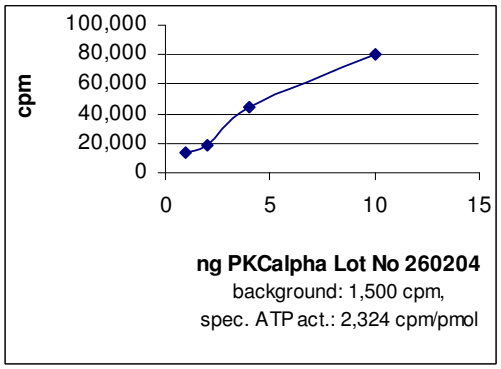
SDS-PAGE analysis



Activity determination



Amino acid sequence information

M13 corresponds to M1 of X52479

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MAISRELVDP NSMADVFPGN DSTASQDVAN RFARKGALRQ KNVHEVKDHK  50
FIARFFKQPT FCSHCTDFIW GFGKQGFQCQ VCCFVVHKRC HEFVTFSCPG 100
ADKGPDTDDP RSQHQFKIHT YGSPTFCDHC GSLLYGLIHQ GMKCDTCDMN 150
VHKQCVINVP SLCGMDHTEK RGRIYLKAEV ADEKLHVTVR DAKNLIPMDP 200
NGLSDPYVKL KLIPDPKNES KQKTKTIRST LNPQWNESFT FKLKPSDKDR 250
RLSVEIWDWD RTTRNDFMGS LSFGVSELMK MPASGWYKLL NQEEGEYYNV 300
PIPEGDEEGN MELRQKFEKA KLGPAGNKVI SPSEDRKQPS NNLDRVKLTD 350
FNFLMVLGKG SFGKVMLADR KGTEELYAIK ILKKDVVIQD DDVECTMVEK 400
RVLALLDKPP FLTQLHSCFQ TVDRLYFVME YVNGGDLMYH IQQVGKFKEP 450
QAVFYAAEIS IGLFFLHKRG IIYRDLKLDN VMLDSEGHIK IADFGMCKEH 500
MMDGVTTRTF CGTPDYIAPE IIAYQPYGKS VDWWAYGVLL YEMLAGQPPF 550
DGEDEDELFQ SIMEHNVSYP KSLSKEAVSI CKGLMTKHPA KRLGCGPEGE 600
RDVREHAFFR RIDWEKLENR EIQPPFKPKV CGKGAENFDK FFTRGQPVLT 650
PPDQLVIANI DQSDFEGFSY VNPQFVHPIL QSAVKLAAAQ LYTRASQPEL 700
APEDPEDLEH HHHHHHH                                     717
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In Vitro Kinase Assay

Assay Components

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**:** PKC peptide (QKRPSQRSKYL), 500 microM
Lipid activator: phosphatidylserine, 0.5 mg/ml; diacylglycerol, 0.5 mg/ml diglyceride, sonicated on ice for 1 minute immediately before use
Protein kinase: PKC, 1 - 5 ng/ microliter diluted in OFAB, directly before use
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 microcentrigue tube
1. Add 10 microliter OFAB
2. Add 10 microliter PKC peptide QKRPSQRSKYL, 500 microM
3. Add 10 microliter PKC enzyme (10 -50 ng per assay)
4. Add 10 microliter lipid activator, freshly prepared
5. Add 10 microliter of the diluted [gamma–^{32}P]ATP
6. Incubate 10 min at 30 ^{o}C.
7. Stop the reaction by setting samples on ice
8. Remove 10 microliter and spot on P81 paper (let bind to the paper for 30 sec)
9. Immerse the paper in 0.75% phosphoric acid, gently shake on a rotator
10. Wash 3 x with phosphoric acid
11. Wash 1 x with acetone
12. Dry under infrared light
13. Read in scintillation counter or Instant Imager

References

Braz J, Gregory K, Pathak A et al (2004) Nature Medicine 10, 248-254
Lahn MM, Sundell KL, Paterson BM (2004) Oncol Rep 11, 151-122
Gutcher I, Webb PR, Anderson NG (2003) Cell Mol Life Sci 60, 1061-1070