KinaseDetect ApS
http://www.kinasedetect.com

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

Certificate of Analysis

Product	**CK2 alpha, Zea mays, active human recombinant, expressed in E. coli crystallization grade**
Cat No	PK-009-01
Lot No	080305-2
Description	Purified recombinant protein kinase CK2 alpha from Zea mays, expressed in E.coli as non fusion protein. The alpha subunit is the catalytic subunit of serine/threonine protein kinase CK2. Constitutively active. Suitable for labeling casein kinase 2 alpha substrates. Shown to be extremely salt sensitive, highest activity without salt. Purified by several chromatography steps (phosphocellulose, Superose 6, Resource Q). M.W. 39,200.

Quality

Protein concentration (Bradford with BSA as standard)	0.7 mg/ml
Purity	> 95% by SDS PAGE
Specific activity (* 1 Unit is defined as 1 picomole phosphate transferred to synthetic peptide (RRRDDDSDDD per min at 37 °C)	1,000,000 Units*/ mg
Protease activity (Twining test)	none

Form**	Liquid. In 25 mM Tris-HCl, 500 mM NaCl, 1 mM DTT, 500 microM PMSF, 50 % glycerol, pH 8.5. ** for crystallization purposes glycerol buffer does not contain glycerol and PMSF
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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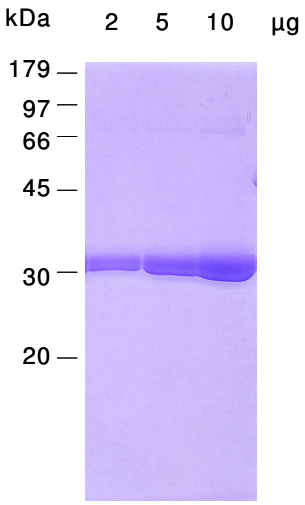
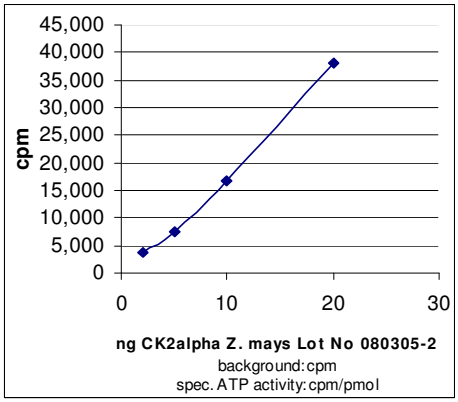
SDS-PAGE analysis



Activity determination



Amino acid sequence information

M1 corresponds to M1 in X61387

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MSKARVYADV NVLRPKEYWD YEALTVQWGE QDDYEVVRKV GRGKYSEVFE 50
GINVNNNEKC IIKILKPVKK KKIKREIKIL QNLCGGPNIV KLLDIVRDQH 100
SKTPSLIFEY VNNTDFKVLY PTLTDYDIRY YIYELLKALD YCHSQGIMHR 150
DVKPHNVMID HELRKLRLID WGLAEFYHPG KEYNVRVASR YFKGPELLVD 200
LQDYDYSLDM WSLGCMFAGM IFRKEPFFYG HDNHDQLVKI AKVLGTDGLN 250
VYLNKYRIEL DPQLEALVGR HSRKPWLKFM NADNQHLVSP EAIDFLDKLL 300
RYDHQERLTA LEAMTHPYFQ QVRAAENSRT RA                    332
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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 DTT, pH 7.5
Substrate**:** CK2 peptide RRRDDDSDDD, 800 microM
Protein kinase**:** Z.m.CK2 alpha, 1 - 10 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)

Assay Procedure

All compounds are pipetted into a microcentrifuge tube on ice
1. Add 10 microliter OFAB
2. Add 10 microliter 800 microM CK2 peptide
3. Add 10 microliter Z.m.CK2alpha (10 - 100 ng/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

References

Boldyreff B, Meggio F, Dobrowolska G, Pinna LA, Issinger OG 1993 Biochim Biophys Acta 1173: 32-38
Dobrowolska G, Boldyreff B, Issinger OG 1991 Biochim Biophys Acta 1129: 139-140

Crystallization conditions according to