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 CK2 (alpha 1)$^{1\text{-}335}$ subunit, active human recombinant, expressed in E. coli crystallization grade**
Cat No	PK-010-01
Lot No	070908

Description

Purified human recombinant CK2 (alpha 1)$^{1\text{-}335}$ expressed in E.coli as a non fusion protein. CK2 (alpha 1)$^{1\text{-}335}$ is a C-terminal deletion mutant comprising amino acids 1-335. Constitutively active. Suitable for labeling CK2alpha substrates. Shown to be extremely salt sensitive, highest activity without salt. Purified by several chromatography steps (phosphocellulose, Resource S, gel filtration).
MALDI-TOF determined M.W. 39,891 (trypsinogen as standard).

CK2 (alpha 1)$^{1\text{-}335}$ is in contrast to the full lenght human CK2 alpha 1 (1-391) stable and suitable for crystallization. The full lenght human recombinant CK2alpha subunit has the tendency to cleave itself leading to two products, i.e. into the full length CK2 alpha with a molecular mass of ca. 44.000 daltons and a polypeptide with a molecular mass of ca. 38.000 daltons. MS analysis revealed the cleavage site and the shortened version CK2 (alpha 1)$^{1\text{-}335}$ was constructed accordingly. The shortened version features a high stability. The function of the missing C-terminal end is unknown. The crystal structure of the C-terminal deletion mutant of human CK2 alpha 1 has been described (Ermakova et al. (2003) J. Mol. Biol. 330, 925-934).

Quality

Protein concentration (Bradford with BSA as standard)	0.27 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 ºC	2,700,000 Units*/ mg
Protease activity (Twining test)	none

Form	Liquid. In 25 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, pH 8.5.
Package size	10 µg
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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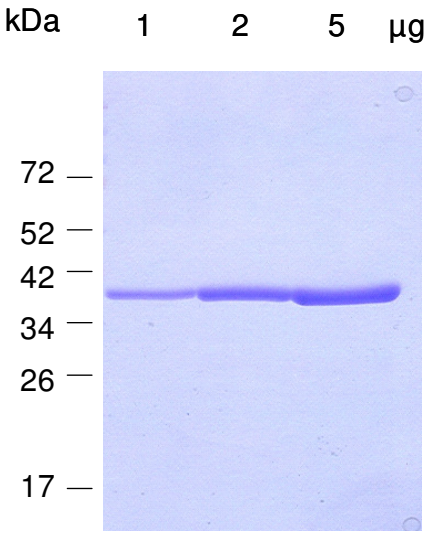
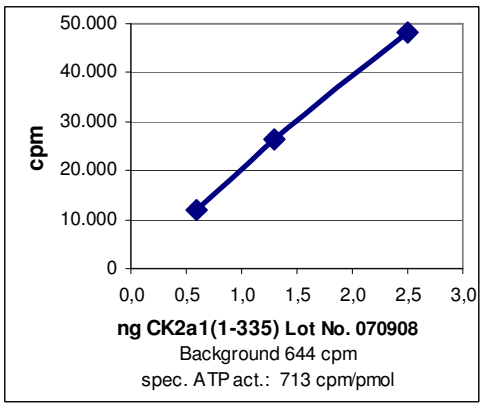
SDS-PAGE analysis



Activity Determination



Amino acid sequence information

M1 corresponds to M1 in M55265 (note: the full length human CK2alpha has 391 amino acids)

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MSGPVPSRAR VYTDVNTHRP REYWDYESHV VEWGNQDDYQ LVRKLGRGKY  50
SEVFEAINIT NNEKVVVKIL KPVKKKKIKR EIKILENLRG GPNIITLADI 100
VKDPVSRTPA LVFEHVNNTD FKQLYQTLTD YDIRFYMYEI LKALDYCHSM 150
GIMHRDVKPH NVMIDHEHRK LRLIDWGLAE FYHPGQEYNV RVASRYFKGP 200
ELLVDYQMYD YSLDMWSLGC MLASMIFRKE PFFHGHDNYD QLVRIAKVLG 250
TEDLYDYIDK YNIELDPRFN DILGRHSRKR WERFVHSENQ HLVSPEALDF 300
LDKLLRYDHQ SRLTAREAME HPYFYTVVKD QARMG                  335
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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**:** CK2alpha 0,25 - 1 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 on ice
1. Add 10 microliter OFAB
2. Add 10 microliter 800 microM CK2 peptide
3. Add 10 microliter hCK2alpha (2.5 - 10 ng/assay)
4. Add 10 microliter of the diluted [gamma–^{32}P]ATP
5. Incubate 10 min at 37 ^{o}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

Reference

Ermakova et al. (2003) J. Mol. Biol. 330, 925-934