KinaseDetect ApS
http://www.kinasedetect.com

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

Certificate of Analysis

Product CK2	**$(\alpha 1)^{1\text{-}335}{}_2\beta_2$ holoenzyme, active human recombinant, expressed in E. coli, crystal grade**
Cat No	PK-027-01
Lot No	140907

Description

Purified recombinant human CK2, subunits alpha 1 (amino acids 1-335) and beta expressed separately as non-fusion proteins in E. coli, reconstituted to the $alpha_2beta_2$ holoenzyme in the course of the purification. Since the CK2 alpha 1 subunit is unstable and prone to degradation, CK2 subunit alpha 1 was shortened at the C-terminal end to obtain the stable, crystallizable $(alpha\ 1)^{1\text{-}335}$ subunit. Constitutively active. Suitable for labeling CK2 substrates. Purified by several chromatography steps (HiTrap Q, Heparin agarose, Superdex 200).
M.W. 135,000.
Approved HUGO gene symbol: CSNK2A1/ CSNK2B
Synonyms: protein kinase CK2, casein kinase 2

Quality

Protein concentration (Bradford with BSA as standard)	0.5 mg/ml
Purity	> 98 % by SDS PAGE
Specific activity	2,390,000 Units*/ mg
(* 1 Unit is defined as 1 picomole phosphate transferred to synthetic peptide (RRRADDSDDDDD per min at 30 °C)	
Protease activity (Twining test)	none

Form Liquid. In 25 mM Tris-HCl, 500 mM NaCl, 1 mM DTT, pH 7.5.

Package size 10 microgram

Storage condition -70 °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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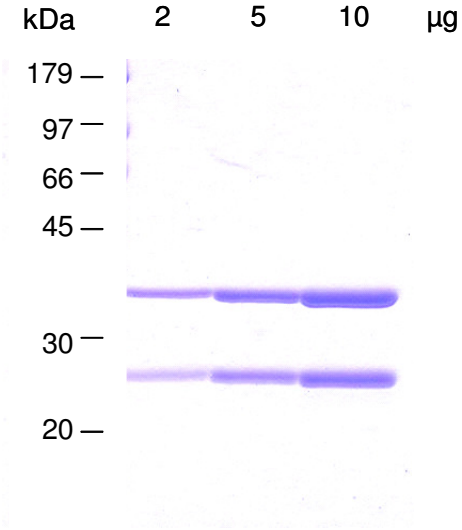
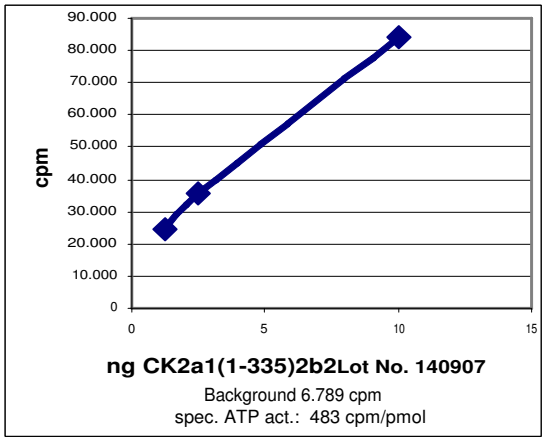
SDS-PAGE analysis



Activity determination



Amino acid sequence information

alpha 1 subunits (amino acids 1-335)
M1 corresponds to M1 in M55265 (note: the full length human CK2 alpha 1 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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beta subunit: M1 corresponds to M1 of X16937

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MSSSEEVSWI SWFCGLRGNE FFCEVDEDYI QDKFNLTGLN EQVPHYRQAL  50
DMILDLEPDE ELEDNPNQSD LIEQAAEMLY GLIHARYILT NRGIAQMLEK 100
YQQGDFGYCP RVYCENQPML PIGLSDIPGE AMVKLYCPKC MDVYTPKSSR 150
HHHTDGAYFG TGFPHMLFMV HPEYRPKRPA NQFVPRLYGF KIHPMAYQLQ 200
LQAASNFKSP VKTIR                                       215
```

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In vitro Kinase Assay

Assay Components

CK2 assay buffer: 75 mM Tris-HCl, 3 mM DTT, 0.5 mg/ml BSA, 300 mM NaCl, pH 8.5
3 M NaCl
Substrate: CK2 peptide RRRADDSDDDDD, 400 microM
Protein kinase: CK2, 2.5 - 40 ng/microliter, diluted in CK2 assay buffer
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 CK2 assay buffer
2. Add 10 microliter 800 microM CK2 peptide
3. Add 10 microliter CK2 (10 - 100 ng/assay)
4. Add 10 microliter of the diluted [gamma–^{32}P]ATP
5. Incubate 10 min at 30 ^{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

References

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Niefind K, Yde CW, Ermakova I, Issinger OG. Evolved to be active: sulfate ions define substrate recognition sites of CK2alpha and emphasise its exceptional role within the CMGC family of eukaryotic protein kinases. **J Mol Biol. 2007** Jul 13;370(3):427-38.

Niefind K, Issinger OG. Primary and secondary interactions between CK2alpha and CK2beta lead to ring-like structures in the crystals of the CK2 holoenzyme. **Mol Cell Biochem. 2005** Jun;274(1-2):3-14.

Yde CW, Ermakova I, Issinger OG, Niefind K. Inclining the purine base binding plane in protein kinase CK2 by exchanging the flanking side-chains generates a preference for ATP as a cosubstrate. **J Mol Biol. 2005** Mar 25;347(2):399-414.

Ermakova I, Boldyreff B, Issinger OG, Niefind K. Crystal structure of a C-terminal deletion mutant of human protein kinase CK2 catalytic subunit. **J Mol Biol. 2003** Jul 25;330(5):925-34.

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Crystallization condition according to Niefind et al. (2001)

CK2 $(\alpha 1)^{1\text{-}335}{}_2\beta_2$ stock solution: 300 mM NaCl, 25 mM Tris-HCl, pH 8.5, 1 mM DTT, 5 mg protein/ml.
3 µl of CK2 stock solution was mixed with 3 µl 2.0 mM $MgCl_2$, 3 µl 1 mM AMPPNP, 1 µl 10 % (w/v) PEG 400 dodecylether and 1 .5 µl reservoir solution composed og 20 % (w/v) PEG 3350, 200 mM K_2HPO_4, pH 9.3. Optimal crystals were grown at 12 ^{o}C.by vapour diffusion in sitting drops.