KinaseDetect ApS
http://www.kinasedetect.com

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

Certificate of Analysis

Product	**CK2 ($\alpha 2)\beta_2$ holoenzyme, active human recombinant**
Cat No	PK-028-01
Lot No	080708

Description

Purified recombinant human CK2, subunits alpha 2 and beta expressed separately, alpha 2 with a N-terminal His-tag in E.coli, beta as non-fusion protein in E.coli, reconstituted to the alpha$_2$beta$_2$ holoenzyme in the course of the purification. Constitutively active. Suitable for labeling CK2 substrates.
Several lines of evidence suggest a trimeric holoenzyme structure consisting of one alpha 2 and two beta molecules, in contrast to the tetrameric holoenzyme structure of a CK2 holoenzyme containing the alpha 1 subunit
Purified by several chromatography steps.
M.W. 95,000.
Approved HUGO gene symbol: CSNK2A2/ CSNK2B
Synonyms: protein kinase CK2, casein kinase 2, CK2$\alpha'\beta_2$

Quality

Protein concentration (Bradford with BSA as standard)	0.3 mg/ml
Purity	> 98 % by SDS PAGE
Specific activity	2,100,000 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, 150 mM NaCl, 1 mM DTT, pH 8.5.

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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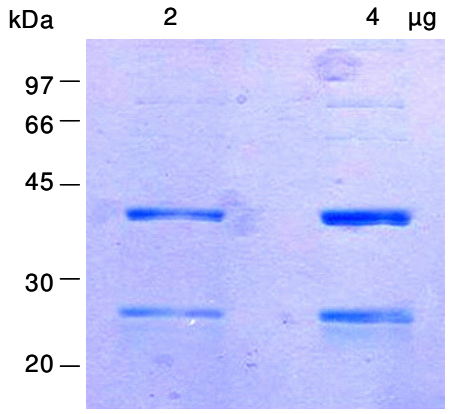
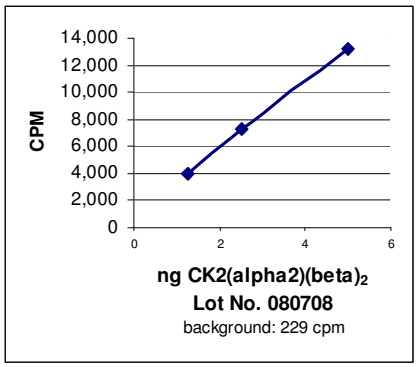
SDS-PAGE analysis



Activity determination



Amino acid sequence information

alpha 2 subunit
M15 corresponds to M1 of NM_001896

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MGSSHHHHHH SQDPMPGPAA GSRARVYAEV NSLRSREYWD YEAHVPSWGN  50
QDDYQLVRKL GRGKYSEVFE AINITNNERV VVKILKPVKK KKIKREVKIL 100
ENLRGGTNII KLIDTVKDPV SKTPALVFEY INNTDFKQLY QILTDFDIRF 150
YMYELLKALD YCHSKGIMHR DVKPHNVMID HQQKKLRLID WGLAEFYHPA 200
QEYNVRVASR YFKGPELLVD YQMYDYSLDM WSLGCMLASM IFRREPFFHG 250
QDNYDQLVRI AKVLGTEELY GYLKKYHIDL DPHFNDILGQ HSRKRWENFI 300
HSENRHLVSP EALDLLDKLL RYDHQQRLTA KEAMEHPYFY PVVKEQSQPC 350
ADNAVLSSGL TAAR                                        364
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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

Assay buffer (AB): 50 mM Tris-HCl, 10 mM NaCl, 10 mM DTT, pH 7.5
Dilution buffer (DB): AB + 0.1 % CHAPS + 600 mM NaCl
Substrate: CK2 peptide RRRADDSDDDDD, 400 microM
Protein kinase: CK2, 0.25 - 1 ng/microliter, diluted in DB
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 AB
3. Add 10 microliter 800 microM CK2 peptide
4. Add 10 microliter CK2 (2.5 - 10 ng/assay)
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 20 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

Olsen BO, Boldyreff B, Niefind K, Issinger OG. Purification and characterization of the CK2α´-based holoenzyme, an isozyme of CK2α: A comparative analysis. Protein Expr Purif 2006, 47, 651-661.

Olsen BB, Rasmussen T, Niefind K, Issinger OG. Biochemical characterization of CK2α and α´ paralogues and their derived holoenzymes: Evidence for the existence of a heterotrimeric CK2α´- holoenzyme forming trimeric complexes. Mol Cell Biochem, 2008 , 316, 37-47.