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 (CSNK2A2), active human recombinant, expressed in E. coli, N-MBP fusion**
Cat No	PK-008-01
Lot No	150506
Description	Purified human recombinant CK2 alpha 2, expressed in E.coli as a N-MBP-fusion protein. The alpha subunit is a catalytic subunit of serine/threonine protein kinase CK2. Constitutively active. Suitable for labeling CK2 alpha substrates. Shown to be extremely salt sensitive, highest activity without salt. Purified by amylose affinity chromatography. Sequence based M.W. 84,297 Approved HUGO gene symbol: CSNK2A2 Synonyms: protein kinase CK2 alpha prime, casein kinase 2 alpha prime

Quality		
	Protein concentration (Bradford with BSA as standard)	0.5 mg/ml
	Purity	> 90 % by SDS PAGE
	Specific activity (* 1 Unit is defined as 1 picomole phosphate transferred to synthetic peptide (RRRDDDSDDD) per min at 37 °C)	2,450,000 Units*/ mg
	Protease activity (Twining test)	none

Form	Liquid. In 20 mM Tris-HCl, 200 mM NaCl, 2 mM DTT, 1 mM EDTA, pH 7.4.
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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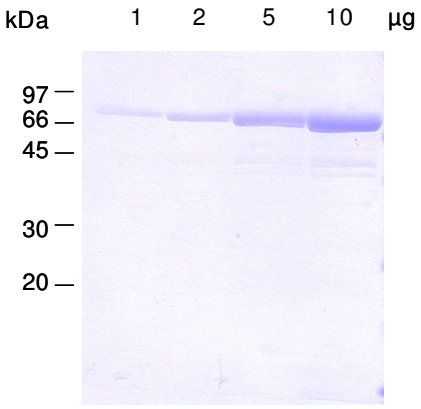
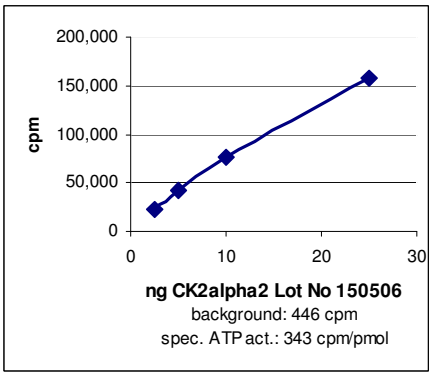
SDS-PAGE analysis



Activity determination



Amino acid sequence information

M394 corresponds to M1 of NM_001896, R743 corresponds to the last amino acid of NM_001869, factor Xa clavage site is underlined

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MKIEEGKLVI WINGDKGYNG LAEVGKKFEK DTGIKVTVEH PDKLEEKFPQ  50
VAATGDGPDI IFWAHDRFGG YAQSGLLAEI TPDKAFQDKL YPFTWDAVRY 100
NGKLIAYPIA VEALSLIYNK DLLPNPPKTW EEIPALDKEL KAKGKSALMF 150
NLQEPYFTWP LIAADGGYAF KYENGKYDIK DVGVDNAGAK AGLTFLVDLI 200
KNKHMNADTD YSIAEAAFNK GETAMTINGP WAWSNIDTSK VNYGVTVLPT 250
FKGQPSKPFV GVLSAGINAA SPNKELAKEF LENYLLTDEG LEAVNKDKPL 300
GAVALKSYEE ELAKDPRIAA TMENAQKGEI MPNIPQMSAF WYAVRTAVIN 350
AASGRQTVDE ALKDAQTNSS SNNNNNNNNN NLGIEGRISE FGSMPGPAAG 400
SRARVYAEVN SLRSREYWDY EAHVPSWGNQ DDYQLVRKLG RGKYSEVFEA 450
INITNNERVV VKILKPVKKK KIKREVKILE NLRGGTNIIK LIDTVKDPVS 500
KTPALVFEYI NNTDFKQLYQ ILTDFDIRFY MYELLKALDY CHSKGIMHRD 550
VKPHNVMIDH QQKKLRLIDW GLAEFYHPAQ EYNVRVASRY FKGPELLVDY 600
QMYDYSLDMW SLGCMLASMI FRREPFFHGQ DNYDQLVRIA KVLGTEELYG 650
YLKKYHIDLD PHFNDILGQH SRKRWENFIH SENRHLVSPE ALDLLDKLLR 700
YDHQQRLTAK EAMEHPYFYP VVKEQSQPCA DNAVLSSGLT AAR        743
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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
Substrate**:** CK2 peptide RRRDDDSDDD, 400 microM
Protein kinase**:** CK2 alpha 0.5 - 2.0 ng/microliter diluted in AB containing 0.05 % Brij 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 AB
2. Add 10 microliter 400 microM CK2 peptide
3. Add 10 microliter CK2 alpha 2 (5 - 20 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

Olsen BB, Boldyreff BS, Niefind K, Issinger OG (2005) Purification and characterization of the CK2alpha'-based holoenzyme, an isozyme of CK2alpha: A comparative analysis. Protein Expression and Purification. Dec 27 (Epub ahead of print)