Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ CK1-alpha1

casein kinase 1 alpha 1

Recombinant Human Active Protein Kinase

HGNC Symbol: CSNK1A1

Synonyms: CK1, CK1a, CK1alpha, CKIa, CKIalpha

Product No.: 0406-0000-1

Lot: 001

Description: Human CK1-alpha1, full length, amino acids M_1-F_{365} (as in NCBI/Protein entry AAY84562.1), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: CK1-alpha1 Lot 001, was confirmed as CK1-alpha1 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 71,434 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

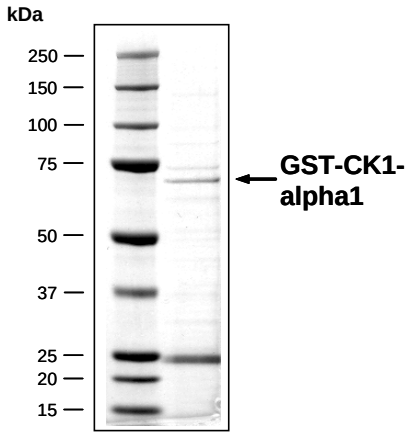
Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.058 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

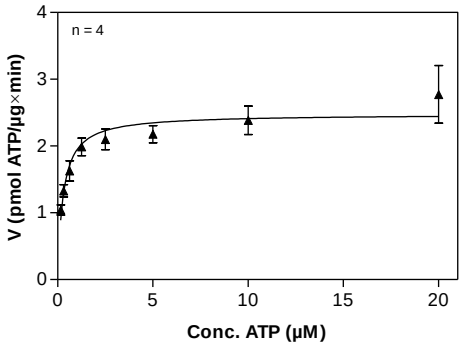
Biochemical Parameters:
Specific kinase activity (P_i transfer): 2.5 pmol/µg × min
ATP-K_M: 0.3 µM

CK1-alpha1 Lot 001:
Coomassie stain



2.0 µg GST-CK1-alpha1

CK1-alpha1 Lot 001:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
• Assay conditions:
60 mM HEPES-NaOH, pH 7.5
3 mM $MgCl_2$
3 mM $MnCl_2$
3 µM Na-orthovanadate
1.2 mM DTT
50 µg / ml $PEG_{20.000}$
ATP (variable)
Substrate: Casein 50 µg/ml
CK1-alpha1: 1 µg/ml
• Filter binding assay
MSFC membrane (Millipore)

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GST-CK1-alpha1 Recombinant Fusion Protein Amino Acid Sequence

1	MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID	60
61	GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV	120
121	DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK	180
181	KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI	240
241	LVPRGSPGLD GIYARGMASS SGSKAEFIVG GKYKLVRKIG SGSFGDIYLA INITNGEEVA	300
301	VKLESQKARH PQLLYESKLY KILQGGVGIP HIRWYGQEKD YNVLVMDLLG PSLEDLFNFC	360
361	SRRFTMKTVL MLADQMISRI EYVHTKNFIH RDIKPDNFLM GIGRHCNKCL ESPVGKRKRS	420
421	MTVSTSQDPS FSGLNQLFLI DFGLAKKYRD NRTRQHIPYR EDKNLTGTAR YASINAHLGI	480
481	EQSRRDDMES LGYVLMYFNR TSLPWQGLKA ATKKQKYEKI SEKKMSTPVE VLCEGFPAEF	540
541	AMYLNYCRGL RFEEAPDYMY LRQLFRILFR TLNHQYDYTF DWTMLKQKAA QQAASSSGQG	600
601	QQAQTPTGKQ TDKTKSNMKG F	660

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: CK1-alpha1

CK1-alpha1 wt[1] Amino Acid Sequence

1	MASSSGSKAE FIVGGKYKLV RKIGSGSFGD IYLAINITNG EEVAVKLESQ KARHPQLLYE	60
61	SKLYKILQGG VGIPHIRWYG QEKDYNVLVM DLLGPSLEDL FNFCSRRFTM KTVLMLADQM	120
121	ISRIEYVHTK NFIHRDIKPD NFLMGIGRHC NKCLESPVGK RKRSMTVSTS QDPSFSGLNQ	180
181	LFLIDFGLAK KYRDNRTRQH IPYREDKNLT GTARYASINA HLGIEQSRRD DMESLGYVLM	240
241	YFNRTSLPWQ GLKAATKKQK YEKISEKKMS TPVEVLCEGF PAEFAMYLNY CRGLRFEEAP	300
301	DYMYLRQLFR ILFRTLNHQY DYTFDWTMLK QKAAQQAASS SGQGQQAQTP TGKQTDKTKS	360
361	NMKGF	420

blue: CK1-alpha1 sequence expressed in recombinant protein

[1]NCBI/Protein accession number AAY84562.1

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