

Certificate of Analysis

CDK4

cyclin dependent kinase 4

Recombinant Human Protein Kinase

HGNC Symbol: CDK4

Synonyms: CMM3, PSK-J3

Product No.: 0142-0000-1

Lot: 002

Description: Human CDK4, amino acids S₄-E₃₀₃ (as in NCBI/Protein entry NP_000066.1), N-terminal GST fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: CDK4, Lot 002, was confirmed as CDK4 by Western Blotting, using a CDK4 specific antibody

Theoretical MW_{Fusion Protein}: 59,700 Da

Expression: Baculovirus infected Sf9 cells

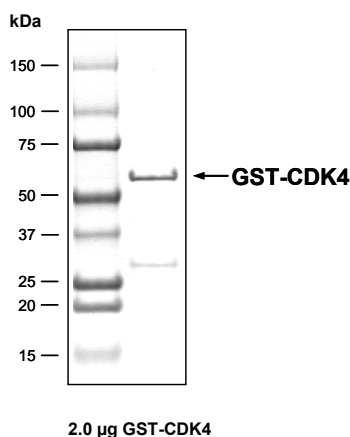
Purification: GST-Affinity Chromatography

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

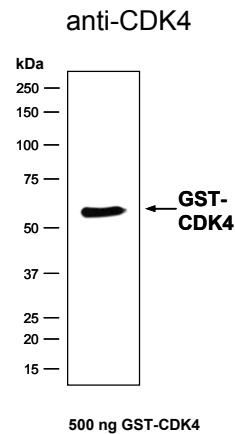
Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!

Protein concentration: 0.300 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

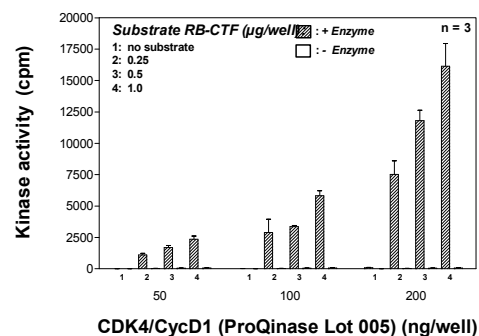
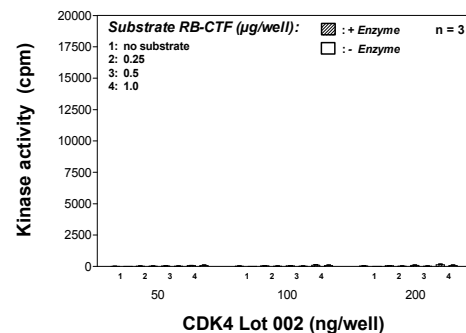
CDK4 Lot 002: Coomassie stain



CDK4 Lot 002: Western Blot Analysis



Kinase activity CDK4 vs active CDK4/CycD1:



Final assay concentrations:

- 60 mM HEPES-NaOH, pH 7.5
- 3 mM MgCl₂
- 3 mM MnCl₂
- 3 µM Na-orthovanadate
- 1.2 mM DTT
- 50 µg / ml PEG_{20,000}
- 1 µM ATP (610,000 cpm ³³P-γ-ATP)
- Substrate (variable): RB-CTF
- Recombinant CDK4 or CDK4/CycD1 (variable)

Assay: ³³PanQinase® Assay

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CoA V1.0 CDK4_Lot002_V3.doc

CDK4

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CDK4 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LVPRGSSRYE	PVAEIGVGAY	240
241	GTVYKARDPH	SGHFVALKSV	RVPNGGGGGG	GLPISTVREV	ALLRRLEAFE	HPNVVRLMDV	300
301	CATSRTDREI	KVTLVFEHVD	QDLRITYLTKA	PPPGLPAETI	KDLMRQFLRG	LDFLHANCIV	360
361	HRDLKPENIL	VTSGGTVKLA	DFGLARIYSY	QMALTPVVVT	LWYRAPEVLL	QSTYATPVDM	420
421	WSVGCIFAEM	FRRKPLFCGN	SEADQLGKIF	DLIGLPPEDD	WPRDVSLPRG	AFPPRGPHPV	480
481	QSVVPEMEES	GAQLLEMLT	FNPHKRISAF	RALQHSYLHK	DEGNPE		540

1-218: GST **Pink: Thrombin** **blue: CDK4 fragment**

CDK4 wt ¹ amino acid sequence							
1	MAT SRYEPVA	EIGVGAYGTV	YKARDPHSGH	FVALKSVRVP	NGGGGGGGLP	ISTVREVALL	60
61	RRLEAFEHPN	VVRLMDVCAT	SRTDREIKVT	LVFEHVDQDL	RTYLDKAPP	GLPAETIKDL	120
121	MRQFLRGLDF	LHANCIVHRD	LKPENILVTS	GGTVKLADFG	LARIYSYQMA	LTPVVVTLWY	180
181	RAPEVLLQST	YATPVMWSV	GCIFAEMFRR	KPLFCGNSEA	DQLGKIFDLI	GLPPEDDWPR	240
241	DVSLPRGAFP	PRGPPVQSV	VPMEESGAQ	LLLEMLTFNP	HKRISAFRAL	QHSYLHKDEG	300
301	NPE						360

blue: CDK4 sequence expressed in fusionprotein **Red:** variant in Fusionprotein

¹NCBI/Protein accession number NP_000066.1

R255H: SNP variation see NCBI/dbSNP ID: rs144657355