

CDK4/CycD1

cyclin dependent kinase 4

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK4

Synonyms: CMM3; PSK-J3

Product No.: 0142-0143-1

Lot: 007

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 and human CycD1, amino acids Q₄-I₂₉₅ (as in [NCBI/Protein](#) entry NP_444284.1), N-terminal GST-fusion protein with a Thrombin cleavage site, coexpressed in Sf9 insect cells

Product identity: CDK4/CycD1 Lot 007, was confirmed as CDK4/CycD1 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{GST-CDK4}: 59,700 Da

Theoretical MW_{GST-CycD1}: 59,995 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

Storage buffer: 50 mM HEPES pH 7.5, 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: 0.314 µg/µl

(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

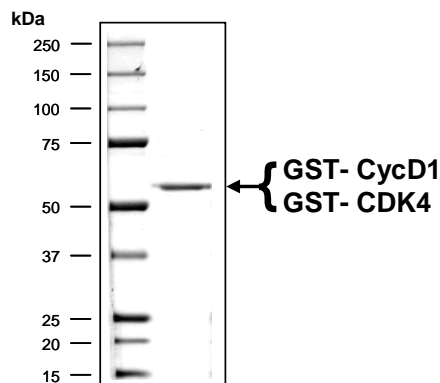
Specific kinase activity (P_i transfer): 99 pmol/µg × min

ATP-K_M: 9.5 µM

Additional assay technology:

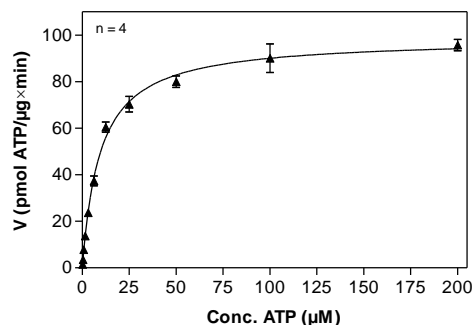
CDK4/CycD1 Lot 007 was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega. ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

CDK4/CycD1 Lot 007: Coomassie stain



2.0 µg CDK4/CycD1 Lot007

CDK4/CycD1 Lot 007: 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 MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg / ml PEG_{20,000}
 - ATP (variable)
 - Substrate: RB-CTF, 100 µg/ml
 - CDK4/CycD1: 4 µg/ml
- Filter binding assay
- MSFC membrane (Millipore)

CDK4/CycD1

Product No.: 0142-0143-1

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

1-218: GST **Pink**: Thrombin cleavage site **blue**: CDK4 **boxed**: variation from RefSeq

CDK4 wt ¹ Amino Acid Sequence							
1	MATSRYPVA	EIGVGAYGTV	YKARDPHSGH	FVALKSVRVP	NGGGGGGLP	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	PRGPRPVQSV	VPEMEESGAQ	LLLEMLTFNP	HKRISAFRAL	QHSYLHKDEG	300
301	NPE						360

blue: CDK4 sequence expressed in recombinant protein **Red**: variant in recombinant protein

¹[NCBI/Protein](#) accession number NP_000066.1

R255H: SNP variation see [NCBI/dbSNP](#) ID: rs144657355

GST-CycD1 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LVPRGSAWIQ	LLCCEVETIR	240
241	RAYPDANLLN	DRVLRAMLKA	EETCAPSVSY	FKCVQKEVLP	SMRKIVATWM	LEVCEEQKCE	300
301	EEVFPLAMNY	LDRFLSLEPV	KKSRLQLLGA	TCMFVASKMK	ETIPLTAEKL	CIYTDNSIRP	360
361	EELLQMEELL	VNKLKWNLAA	MTPHDFIEHF	LSKMPEAEEN	KQIRKHAQT	FVALCATDVK	420
421	FISNPPSMVA	AGSVAAVQG	LNLRSNNFL	SYRRLTRFLS	RVIKCDPDCL	RACQEQIEAL	480
481	LESSLRQAQQ	NMDPKAAEEE	EEEEEEVDLA	CTPTDVRD	I		540

1-218: GST **Pink**: Thrombin cleavage site **blue**: CycD1

CycD1 wt ² Amino Acid Sequence							
1	MEHQLLCCEV	ETIRRAYPDA	NLLNDRVLR	MLKAEETCAP	SVSYFKCVQK	EVLPSMRKIV	60
61	ATWMLEVCEE	QKCEEEVFPL	AMNYLDRFLS	LEPVKKSRLQ	LLGATCMFVA	SKMKETIPLT	120
121	AEKLCIYTDN	SIRPEELLQ	ELLVNKLKW	NLAAMTPHDF	IEHFLSKMPE	AEENKQIIRK	180
181	HAQTFVALCA	TDVCFISNPP	SMVAAGSVVA	AVOGLNLRSP	NNFLSYRILT	RFLSRVIKCD	240
241	PDCLRACQEQ	IEALLESSLR	QAQQNMDPKA	AEEEEEEEEE	VDLACTPTDV	RDVDI	300

blue: CycD1 sequence expressed in recombinant protein

²[NCBI/Protein](#) accession number NP_444284.1

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