

CDK4/CycD3

cyclin dependent kinase 4

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK4

Synonyms: CMM3; PSK-J3

Product No.: 0142-0373-1

Lot: 001

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 CycD3, amino acids M₁-L₂₉₂ (as in [NCBI/Protein](#) entry NP_001751.1), N-terminal HIS6-fusion protein with a Thrombin cleavage site, coexpressed in Sf9 insect cells

Product identity: CDK4/CycD3 Lot 001, was confirmed as CDK4/CycD3 by mass spectroscopy LC-ESI-MS/MS

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

Theoretical MW_{CycD3}: 37,260 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: 0.337 µg/µl

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

Biochemical Parameters:

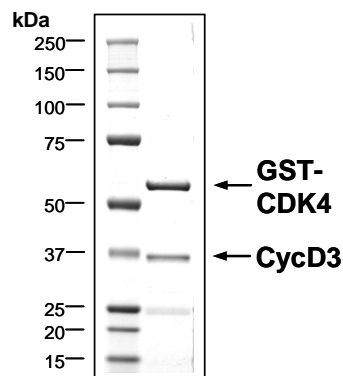
Specific kinase activity (P_i transfer): 75 pmol/µg × min

ATP-K_M: 18.7 µM

Additional assay technology:

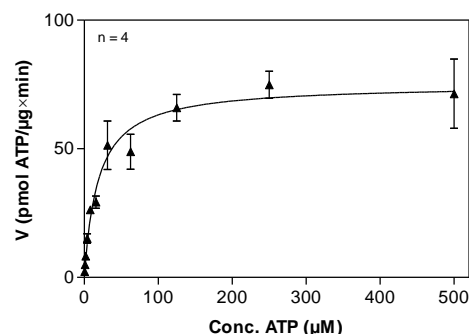
CDK4/CycD3 LOT001 was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega. ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

CDK4/CycD3: Coomassie stain



2.0 µg GST-CDK4/CycD3

CDK4/CycD3: 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, 200 µg/ml
 - CDK4/CycD3: 4 µg/ml
- Filter binding assay
- MSFC membrane (Millipore)

CDK4/CycD3

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GST-CDK4 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLIERDEGDK	WRNKKFELGL	EFNLPYYID	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	AFPPRGP	480
481	QSVVPEMEES	GAQLLEMLT	FNP HKRISAF	RALQHSYLHK	DEGNPE		540

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: CDK4 **boxed**: variation from RefSeq

CDK4 wt ¹ Amino Acid Sequence							
1	MATSRYPVA	EIVGAYGTV	YKARDPHSGH	FVALKSVRVP	NGGGGGGLP	ISTVREVALL	60
61	RRLEAFEHPN	VVRLMDVCAT	SRTDREIKVT	LVFEHVDQDL	RTYLDKAPP	GLPAETIKDL	120
121	MRQFLRGLDF	LHANCIVHRD	LKPENILVTS	GGTVKLADFG	LARIYSYQMA	LTPVVVTLWY	180
181	RAPEVLLQST	YATPVDMWSV	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

¹NCBI/Protein accession number NP_000066.1

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

GST-Kinase Recombinant Fusion Protein Amino Acid Sequence							
1	MSPIDPMGHH	HHHHGRRRAS	VAAGILVPRG	SPGLDGIYAR	GIQASMELLC	CEGTRHAPRA	60
61	GPDPRL LGDQ	RVLQSLRLLE	ERYVPRASYF	QCVQREIKPH	MRKMLAYWML	EVCEEQRCEE	120
121	EVFPLAMNYL	DRYLSCVPTR	KAQLQLLGAV	CMLLASKLRE	TPPLTIEKLC	IYTDHAVSPR	180
181	QLRDWEVLVL	GKIKWDLAAV	IAHDFLAFIL	HRLSLPRDRQ	ALVKKHAQTF	LALCATDYTF	240
241	AMYPPSMIAT	GSIGAAVQGL	GACSMGDEL	TELLAGITGT	EVDCLRACQE	QIEAALRESL	300
301	REAQTSSSP	APKAPRGSSS	QGPSQTSTPT	DVTAIHL			360

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: CycD3 **boxed**: variation from RefSeq

CycD3 wt ² Amino Acid Sequence							
1	MELLCEGTR	HAPRAGPDPR	LLGDQRLVQS	LLRLEERYVP	RASYFQCVQR	EIKPHMRKML	60
61	AYWMLEVCEE	QRCEEEVFPL	AMNYLDRYLS	CVPTRKAQLQ	LLGAVCMLLA	SKLRETTPLT	120
121	IEKLCIYTDH	AVSPRQLRDW	EVLVLGKLLK	DLAAVIAHDF	LAFILHRLSL	PRDRQALVKK	180
181	HAQTFLALCA	TDYTFAMYPP	SMIATGSIGA	AVQGLGACSM	SGDELTELLA	GITGTEVDCL	240
241	RACQE QIEAA	LRESLREASQ	TSSSPAPKAP	RGSSSQGPSQ	TSTPTDVTAI	HL	300

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

²NCBI/Protein accession number NP_001751.1

S259A: SNP variation see [NCBI/dbSNP](#) ID: rs386700585

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