

ProQinase™ CDK4/CycD2

cyclin dependent kinase 4

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK4

Synonyms: CMM3; PSK-J3

Product No.: 0142-0375-1

Lot: 005

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 CycD2, amino acids M₁-L₂₈₉ (as in [NCBI/Protein](#) entry NP_001750.1), N-terminally fused to HIS₆-Thrombin cleavage site, coexpressed in Sf9 insect cells

Product identity: CDK4/CycD2 Lot 005, was confirmed as CDK4/CycD2 by mass spectroscopy LC-ESI-MS/MS

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

Theoretical MW_{CycD2}: 37,824 Da

Expression host: Sf9 insect cells

Purification: Immobilized Metal Affinity Chromatography

Activation: in vitro by CAK1

Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 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: 0.213 µg/µl

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

Biochemical Parameters:

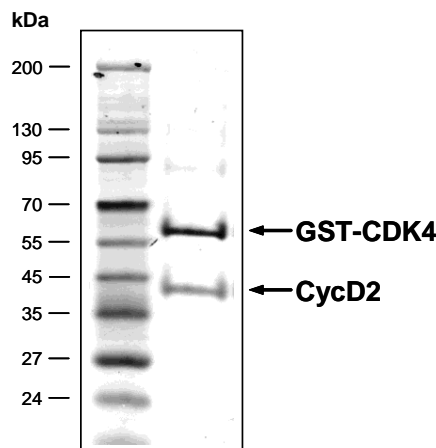
Specific kinase activity (P_i transfer): 141 pmol/µg × min

ATP-K_M: 3.8 µM

Additional assay technology:

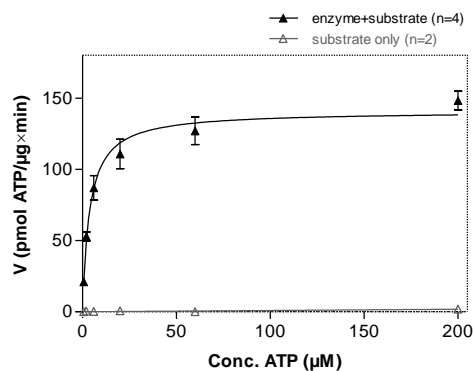
CDK4/CycD2 Lot 005 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

**CDK4/CycD2 Lot 005:
Coomassie stain**



2.0 µg CDK4/CycD2

**CDK4/CycD2 Lot 005:
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/CycD2: 2 µg/ml
- Filter binding assay
- MSFC membrane (Millipore)

ProQinase™ CDK4/CycD2

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GST-CDK4 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDKVLTQSM	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	QDLRTRYLDKA	PPPGLPAETI	KDLMRQFLRG	LDFLHANCIV	360
361	HRDLKPENIL	VTSGGTVKLA	DFGLARIYSY	QMALTPVVVT	LWYRAPEVLL	QSTYATPVDM	420
421	WSVGCIFAEM	FRRKPLFCGN	SEADQLGKIF	DLIGLPPEDD	WPRDVS LPRG	AFPPRGPHPV	480
481	QSVVPEMEES	GAQLLLEMLT	FNPHKRISAF	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	EIGVGAYGTV	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

HIS-CycD2 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPIDPMGHH	HHHHGRRRAS	VAAGILVPRG	SPGLDGIYAR	GIQASMELLC	HEVDPVRRAV	60
61	RDRNLLRDDR	VLQNLITIEE	RYLPQCSYFK	CVQKDIQPYM	RRMVATWMLE	VCEEQKCEE	120
121	VFPLAMNYLD	RFLAGVPTPK	SHLQLLGAVC	MFLASKLKET	SPLTAEKLCI	YTDNSIKPQE	180
181	LLEWELVVLG	KLKWNLA AVT	PHDFIEHILR	KLPQOREKLS	LIRKHAQTFI	ALCATDFKFA	240
241	MYPPSMIATG	SVGAAICGLQ	QDEEVSSLTC	DALTELLAKI	TNTDVDCLKA	CQEIQIEAVLL	300
301	NSLQOYRQDQ	RDGSKSEDEL	DQASTPTDVR	DIDL			360

Red: HIS6-tag Pink: Thrombin cleavage site blue: CycD2

CycD2 wt ² Amino Acid Sequence							
1	MELLCHEVDP	VRAVRDRNL	LRDDRVLQNL	LTIIEERYLPQ	CSYFKCVQKD	IQPYMRMVA	60
61	TWMLEVCEEQ	KCEEEVFLA	MNYLDRFLAG	VPTPKSHLQL	LGAVCMFLAS	KLKETSPLTA	120
121	EKLCIYTDNS	IKPQELLEWE	LVVLGKWKWN	LA AVTPHDFI	EHILRKL PQ	REKLSLIRKH	180
181	AQTFIALCAT	DFKFAMYPPS	MIATGSV GAA	ICGLQODEEV	SSLTCDALTE	LLAKITNTDV	240
241	DCLKACQEIQI	EAVLLNSLQQ	YRQDQRD GSK	SEDEL DQAST	PTDVRDIDL		300

blue: CycD2 sequence expressed in recombinant protein

²NCBI/Protein accession number NP_001750.1

HGNC identifier CycD2: CCND2

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