Certificate of Analysis

Recombinant Proteins

ProQinase™ 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 S$_4$-E$_{303}$ (as in NCBI/Protein entry NP_000066.1), N-terminal GST-fusion protein with a Thrombin cleavage site and human CycD3, amino acids M$_1$-L$_{292}$ (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

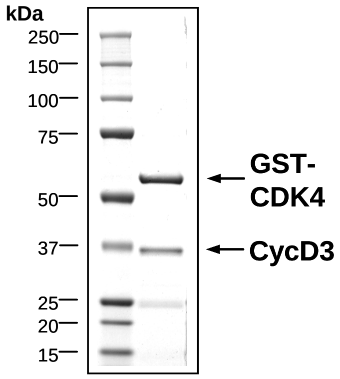
Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

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)

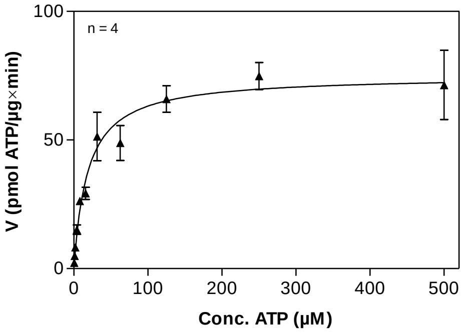
Biochemical Parameters:
Specific kinase activity (P$_i$ transfer): 75 pmol/µg × min
ATP-K$_M$: 18.7 µM

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 MgCl$_2$
  3 mM MnCl$_2$
  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)

Additional assay technology:
CDK4/CycD3 LOT001 was also successfully tested by Reaction Biology for the use with the ADP-Glo^{TM} Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

This product was manufactured at Reaction Biology in Freiburg, Germany, and is for in vitro research use only, not for use in humans or animals.

ProQinase™ CDK4/CycD3

Product No.: 0142-0373-1

GST-CDK4 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 **LVPRGSSRYE PVAEIGVGAY**	240
241	**GTVYKARDPH SGHFVALKSV RVPNGGGGGG GLPISTVREV ALLRRLEAFE HPNVVRLMDV**	300
301	**CATSRTDREI KVTLVFEHVD QDLRTYLDKA PPPGLPAETI KDLMRQFLRG LDFLHANCIV**	360
361	**HRDLKPENIL VTSGGTVKLA DFGLARIYSY QMALTPVVVT LWYRAPEVLL QSTYATPVDM**	420
421	**WSVGCIFAEM FRRKPLFCGN SEADQLGKIF DLIGLPPEDD WPRDVSLPRG 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	MAT**SRYEPVA EIGVGAYGTV YKARDPHSGH FVALKSVRVP NGGGGGGGLP ISTVREVALL**	60
61	**RRLEAFEHPN VVRLMDVCAT SRTDREIKVT LVFEHVDQDL RTYLDKAPPP 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

[1] NCBI/Protein accession number NP_000066.1
R255H: SNP variation see NCBI/dbSNP ID: rs144657355

GST-Kinase Recombinant Fusion Protein Amino Acid Sequence

1	MSPIDPMG**HH HHHH**GRRRAS VAAGI**LVPRG S**PGLDGIYAR GIQAS**MELLC CEGTRHAPRA**	60
61	**GPDPRLLGDQ RVLQSLLRLE ERYVPRASYF QCVQREIKPH MRKMLAYWML EVCEEQRCEE**	120
121	**EVFPLAMNYL DRYLSCVPTR KAQLQLLGAV CMLLASKLRE TTPLTIEKLC IYTDHAVSPR**	180
181	**QLRDWEVLVL GKLKWDLAAV IAHDFLAFIL HRLSLPRDRQ ALVKKHAQTF LALCATDYTF**	240
241	**AMYPPSMIAT GSIGAAVQGL GACSMSGDEL TELLAGITGT EVDCLRACQE QIEAALRESL**	300
301	**REAAQTSSSP 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	**MELLCCEGTR HAPRAGPDPR LLGDQRVLQS LLRLEERYVP RASYFQCVQR EIKPHMRKML**	60
61	**AYWMLEVCEE QRCEEEVFPL AMNYLDRYLS CVPTRKAQLQ LLGAVCMLLA SKLRETTPLT**	120
121	**IEKLCIYTDH AVSPRQLRDW EVLVLGKLKW DLAAVIAHDF LAFILHRLSL PRDRQALVKK**	180
181	**HAQTFLALCA TDYTFAMYPP SMIATGSIGA AVQGLGACSM SGDELTELLA GITGTEVDCL**	240
241	**RACQEQIEAA LRESLREASQ TSSSPAPKAP RGSSSQGPSQ TSTPTDVTAI HL**	300

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

[2] NCBI/Protein accession number NP_001751.1
S259A: SNP variation see NCBI/dbSNP ID: rs386700585