

ProKinase™ CDK6/CycD2

cyclin-dependent kinase 6

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK6

Synonyms: PLSTIRE

Product No.: 0051-0375-1

Lot: 005

Description: Human CDK6, amino acids M₁-A₃₂₆ (as in [NCBI/Protein](#) entry NP_001250.1), 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: CDK6/CycD2 Lot 005, was confirmed as CDK6/CycD2 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{GST-CDK6}: 63,279 Da

Theoretical MW_{CycD2}: 37,823 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: 0.210 µg/µl

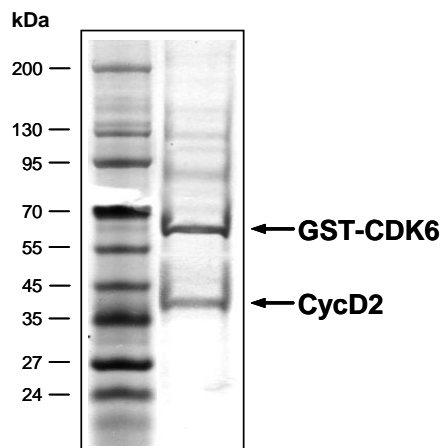
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 126 pmol/µg × min

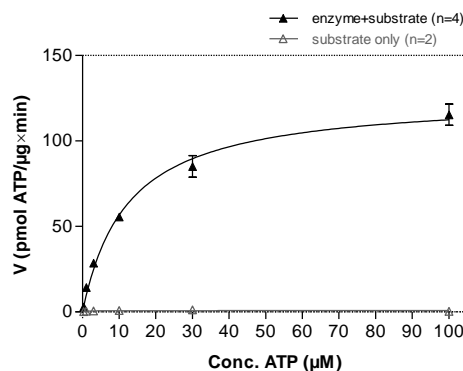
ATP-K_M: 12 µM

CDK6/CycD2 Lot 005:
Coomassie stain



2.0 µg CDK4/CycD2

CDK6/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
 - Kinase: 4 µg/ml
- Filter binding assay
- MSFC membrane (Millipore)

Additional assay technology:

CDK6/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

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ProQinase™ CDK6/CycD2

Product No.: 0051-0375-1

GST-CDK6 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDKVLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LVPRGSMKED	GLCRADQQYE	240
241	CVAEIGEGAY	GKVFKARDLK	NGGRFVALKR	VRVQTGEEGM	PLSTIREVAV	LRHLETFEHP	300
301	NVRLFDVCT	VSRTDRETKL	TLVFEHVDQD	LTTYLDKVE	PGVPTETIKD	MMFQLLRGLD	360
361	FLHSHRVVHR	DLKPQNILVT	SSGQIKLADF	GLARIYSFQM	ALTSVVVTLW	YRAPEVLLQS	420
421	SYATPVDLWS	VGCIFAEMFR	RKPLFRGSSY	VDQLGKILDV	IGLPGEEDWP	RDVALPRQAF	480
481	HSKSAQPIEK	FVTDIDELGK	DLLLKCLTFN	PAKRISAYSA	LSPYFQDLE	RCKENLDSHL	540
541	PPSQNTSELN	TA					600

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: CDK6 **boxed**: variation from RefSeq

CDK6 wt ¹ Amino Acid Sequence							
1	MEKDGLCRAD	QQYECVAEIG	EGAYGKVFKA	RDLKNGGRFV	ALKRVRVQTG	EEGMPLSTIR	60
61	EVAVLRHLET	FEHPNVVRLF	DVCTVSRTDR	ETKLTLVFEH	VDQDLTTYLD	KVPEPGVPTE	120
121	TIKDMMFQLL	RGLDFLHSHR	VVHRDLKPQN	ILVTSSGQIK	LADFGRLARIY	SFQMALTSVV	180
181	VTLWYRAPEV	LLQSSYATPV	DLWSVGCIFA	EMFRRKPLFR	GSSVDVQLGK	ILDVIGLPEE	240
241	EDWPRDVALP	RQAFHSKSAQ	PIEKFVTDID	ELGKDLLLKC	LTFNPAKRIS	AYSALSHPHYF	300
301	QDLERCKENL	DSHLPSSQNT	SELNTA				360

blue: CDK6 sequence expressed in recombinant protein **Red**: variant in recombinant protein

¹[NCBI/Protein](#) accession number NP_001250.1

HIS-CycD2 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPIDPMG HH	HHHH GRRRAS	VAAGI LVPRG	SPGLDGIYAR	GIQAS MELLC	HEVDPVRRAV	60
61	RDRNLLRDDR	VLQNLTLTIEE	RYLPQCSYFK	CVQKDIQPYM	RRMVATWMLE	VCEEQKCEEE	120
121	VFPLAMNYLD	RFLAGVPTPK	SHLQLLGAVC	MFLASKLKET	SPLTAEKLCI	YTDNSIKPQE	180
181	LLEWELVVLG	KLKWNLAAVT	PHDFIEHILR	KLPQREKLS	LIRKHAQTFI	ALCATDFKFA	240
241	MYPPSMIATG	SVGAAICGLQ	QDEEVSSLTC	DALTELLAKI	TNTDVCCLKA	CQEQIEAVLL	300
301	NSLQQYRQDQ	RDGSKSEDEL	DQASTPTDVR	DIDL			360

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: CycD2

CycD2 wt ² Amino Acid Sequence							
1	MELLCHEVDP	VRRAVRDRNL	LRDDRVLQNL	LTIIEERYLPQ	CSYFKCVQKD	IQPYMRRMVA	60
61	TWMLEVCEEQ	KCEEEVFPLA	MNYLDRFLAG	VTPKSHLQL	LGAVCMFLAS	KLKETSPLTA	120
121	EKLCIYTDNS	IKPQELLEWE	LVVLGKWKWN	LAAVTPHDFI	EHILRKLQQ	REKLSLIRKH	180
181	AQTFIALCAT	DFKFAMYPPS	MIATGSVGAA	ICGLQDDEEV	SSLTCDALTE	LLAKITNTDV	240
241	DCLKACQEIQ	EAVLLNSLQQ	YRQDQRDGSK	SEDELDQAST	PTDVRDIDL		300

blue: CycD2 sequence expressed in recombinant protein

²[NCBI/Protein](#) accession number NP_001750.1
HGNC identifier CycD2: CCND2