

ProQinase™ CDK6/CycD1 (non activated)

Cyclin dependent kinase 6

Recombinant Human Protein Kinase

HGNC Symbol: CDK6

Synonyms: PLSTIRE; cell division protein kinase 6

Product No.: 0051-0143-1

Lot: 004

Description: Coexpression of human CDK6, full length, amino acids M₁-A₃₂₆ (NCBI/Protein entry NP_001250.1), N-terminally fused to GST-Thrombin cleavage site and human CycD1, full length, amino acids Q₄-I₂₉₅ (NCBI/Protein entry NP_444284.1), N-terminally fused to GST-Thrombin cleavage site, expressed in Sf9 insect cells

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

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

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by CAK1 in-vitro activation

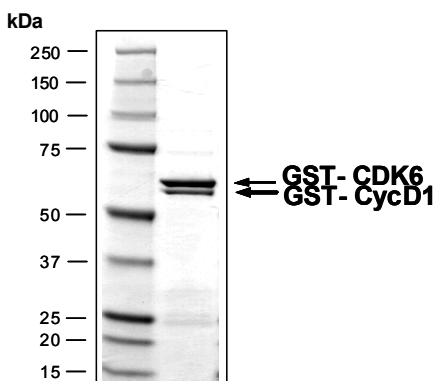
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

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!

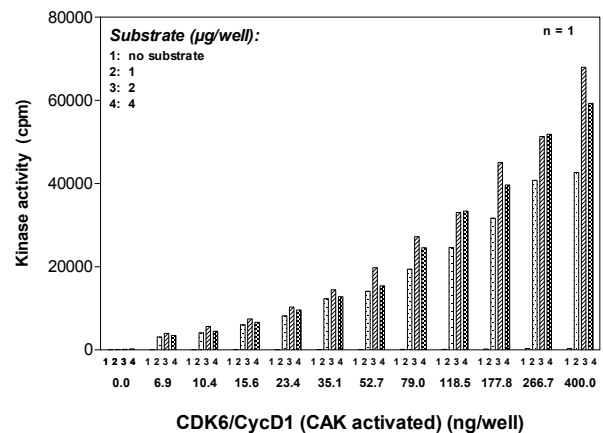
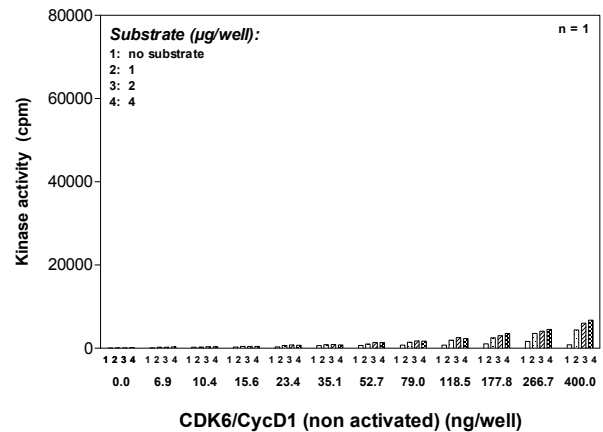
Protein concentration: 0.388 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

CDK6/CycD1 (non activated) Lot 004:
Coomassie stain



2.0 µg GST-CDK6/GST-CycD1 (non activated)

Kinase activity CDK6/CycD1 (non activated) vs CDK6 CycD1 (CAK activated):



Final assay concentrations:

- 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}
- 1 µM ATP
- Substrate: RBER-CHKtide, 20µg/ml
- Kinase: CDK6/CycD1 (non activated) or CDK6/CycD1 (activated) [1µg/ml]

Assay: ³³PanQinase® Assay

Recombinant Proteins

ProQinase™ CDK6/CycD1 (non activated)

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

1-218: GST 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	DVCTVSRTRD	ETKLTLVFEH	VDQLTTYLD	KVPEPGVPT	120
121	TIKDMMFQLL	RGLDFLHSHR	VVHRDLKPQN	ILVTSSGQIK	LADFGIARIY	SFQMALTSV	180
181	VTLWYRAPEV	LLQSSYATPV	DLWSVGCIFA	EMFRRKPLFR	GSSDQDQLGK	ILDVIGLPGE	240
241	EDWPRDVALP	RQAFHKSQAQ	PIEKFVTDID	ELGKDLLLKC	LTFNPAKRIS	AYSALSHPYF	300
301	QDLERCKENL	DSHLPPSQNT	SELNTA				360

blue: CDK6 sequence expressed in fusionprotein Red: variant in fusionprotein

¹NCBI/Protein accession number NP_001250.1

CycD1 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LVPRGS	AWIQ	240
241	RAYPDANLLN	DRVLRAMLKA	EETCAPSVSY	FKCVQKEVLP	SMRKIVATWM	LEVCEEQKCE	300
301	EEVFPLAMNY	LDRFLSLEPV	KKSRLQLLGA	TCMFVASKMK	ETIPLTAEKL	CIYTDNSIRP	360
361	EELLQMELLL	VNKLKWNLAA	MTPHDFIEHF	LSKMPEAEEN	KQIRKHAQT	FVALCATDVK	420
421	FISNPPSMVA	AGSVVAAVQG	LNLRSNNFL	SYRLTRFLS	RVIKCDPDCL	RACQEIQEAL	480
481	LESSLRQAQQ	NMDPKAAEEE	EEEEEEVDLA	CTPTDVRDVD	I		540

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

CycD1 wt ¹ amino acid sequence							
1	MEHQLLCCEV	ETIRRAYPDA	NLLNDRVLR	MLKAEETCAP	SVSYFKCVQK	EVLPSMRKIV	60
61	ATWMLEVCEE	QKCEEEVFPL	AMNYLDRELS	LEPVKKSRLQ	LLGATCMFVA	SKMKETIPLT	120
121	AEKLCIYTDN	SIRPEELLQM	ELLLVNKLKW	NLAAMTPHDF	IEHFLSKMPE	AEENKQIRK	180
181	HAQTFVALCA	TDVKFISNPP	SMVAAGSVVA	AVQGLNLRSP	NNFLSYRLT	RFLSRVIKCD	240
241	PDCLRACQEQ	IEALLESSLR	QAQQNMDPKA	AEEEEEEEEE	VDLACTPTDV	RDVDI	300

blue: CycD1 sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_444284.1