

Certificate of Analysis

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

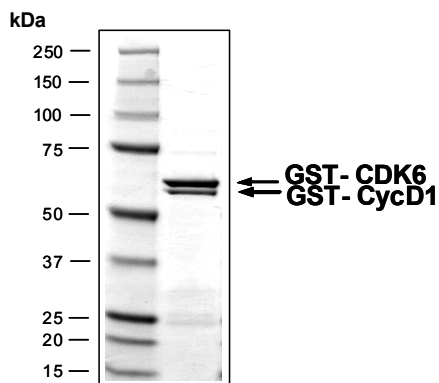
Storage temperature: -80°C

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)

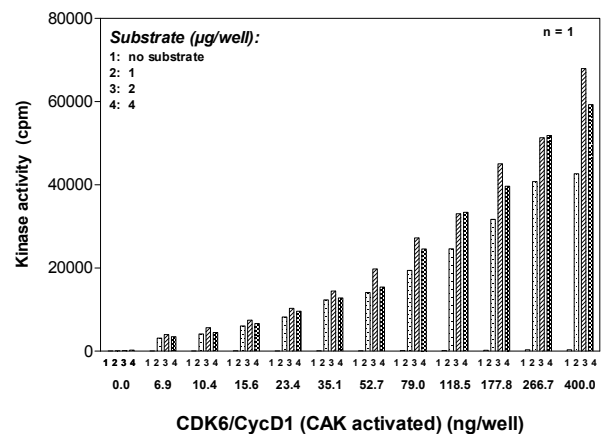
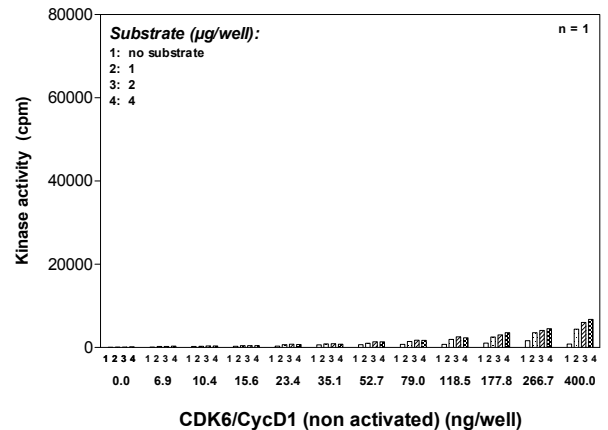
CDK6/CycD1 (non activated) Lot 004:

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

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CDK6/CycD1 (non activated)

Product No.: 0051-0143-1

CDK6 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKH	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LVPRGSM	GLCRADQQYE	240
241	CVAEIGEGAY	GKVFKARDLK	NGGRFVALKR	VRVQTGEEGM	PLSTIREVAV	LRHLETFEHP	300
301	NVRLFDVCT	VSRTDRETKL	TLVFEHVDQD	LTTYLDKVPE	PGVPTETIKD	MMFQLLRGLD	360
361	FLHSHRVVHR	DLKPQNILVT	SSGQIKLADF	GLARIYSFQM	ALTSVVVTLW	YRAPEVLLQS	420
421	SYATPVDLWS	VGCIFAEMFR	RKPLFRGSSY	VDQLGKILDV	IGLPGEEDWP	RDVALPRQAF	480
481	HSKSAQPIEK	FVTDIDELGK	DLLLKCLTFN	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	DVCTVSRTDR	ETKLTLVFEH	VDQLTITYLD	KVPEPGVPTE	120
121	TIKDMMFQLL	RGLDFLHSHR	VVHRDLKPQN	ILVTSSGQIK	LADFLARIY	SFQMALTSVV	180
181	VTLWYRAPEV	LLQSSYATPV	DLWSVGCIFA	EMFRRKPLFR	GSSVDVQLGK	ILDVIGLPGE	240
241	EDWPRDALP	RQAFHSKSAQ	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	LEYLEEKYE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKH	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LVPRGSAWIQ	LLCCEVETIR	240
241	RAYPDANLLN	DRVLRAMLKA	EETCAPSVSY	FKCVQKEVLP	SMRKIVATWM	LEVCEEQKCE	300
301	EEVFPLAMNY	LDRFLSLEPV	KKSRLQLLGA	TCMFVASKMK	ETIPLTAEKL	CIYTDNSIRP	360
361	EELLQPELLL	VNKLKWNLAA	MPHDFIEHF	LSKMPEAEEN	KQIIRKHAQT	FVALCATDVK	420
421	FISNPPSMVA	AGSVAAVQ	LNLRSNNFL	SYRRLTRFLS	RVIKCDPDCL	RACQEIQIEAL	480
481	LESSLRQAQQ	NMDPKAAEEE	EEEEEEVDLA	CTPTDVRDVI			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	AMNYLDRFLS	LEPVKKSRLQ	LLGATCMFVA	SKMKETIPLT	120
121	AEKLCIYTDN	SIRPEELLQM	ELLLVNKLKW	NLAAMTPHDF	IEHFLSKMPE	AEENKQIIRK	180
181	HAQTFVALCA	TDVKFISNPP	SMVAAGSVVA	AVQGLNLRSP	NNFLSYRRLT	RFLSRVIKCD	240
241	PDCLRACQEQ	IEALLESLR	QAQQNMDPKA	AEAAAAAAAA	VDLACTPTDV	RDVDI	300

blue: CycD1 sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_444284.1

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