

Certificate of Analysis

CDK6

cyclin dependent kinase 6

Recombinant Human Protein Kinase

HGNC Symbol: CDK6

Synonyms: PLSTIRE

Product No.: 0051-0000-1

Lot: 002

Description: Human CDK6, amino acids M₁-A₃₂₆ (as in NCBI/Protein entry NP_001250.1), N-terminal GST-fusion protein, expressed in Sf9 insect cells

Product identity: CDK6, Lot 002, was confirmed as CDK6 by Western Blotting, using a CDK6 specific antibody

Theoretical MW_{Fusion Protein}: 63,279 Da

Expression: Baculovirus infected Sf9 cells

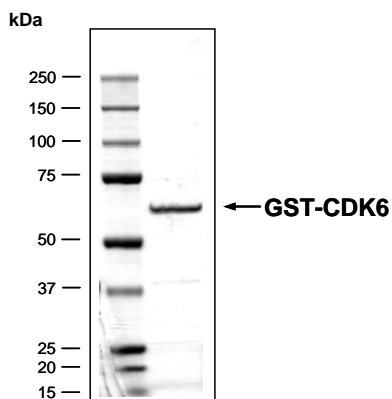
Purification: GST-Affinity Chromatography

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

Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!

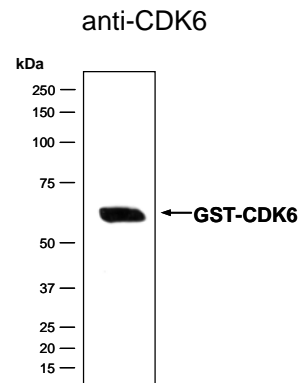
Protein concentration: 0.620 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

CDK6 Lot 002: Coomassie stain



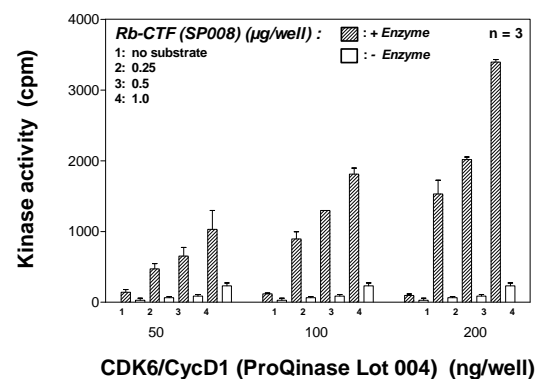
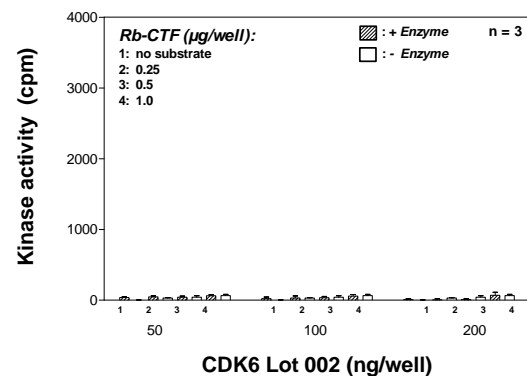
2.0 µg GST-CDK6

CDK6 Lot 002: Western Blot Analysis



500 ng GST-CDK6

Kinase activity CDK6 vs active CDK6/CycD1:



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 (610,000 cpm ³³P-γ-ATP)
- Substrate (variable): RB-CTF
- Recombinant CDK6 or CDK6/CycD1 (variable)

Assay: ³³PanQinase® Assay

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CcA V1.0 CDK6_Lot002_V1.doc

CDK6

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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	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LVPRGSMEKD	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** **blue: CDK6 fragment**

CDK6 wt ¹ amino acid sequence							
1	MEKDGLCRAD	QQYECVAEIG	EGAYGKVFKA	RDLKNGGRFV	ALKRVRVQTG	EEGMPLSTIR	60
61	EVAVLRHLET	FEHPNVVRLF	DVCTVSRTDR	ETKLTLVFEH	VDQDLTTYLD	KVPEPGVPTE	120
121	TIKMMFQLL	RGLDFLHSHR	VVHRDLKPQN	ILVTSSGQIK	LADFGIARIY	SFQMALTSVV	180
181	VTLWYRAPEV	LLQSSYATPV	DLWSVGCIFA	EMFRRKPLFR	GSSD¹VDQLGK	ILDVIGLPGE	240
241	EDWPRDVALP	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