

Certificate of Analysis

CDK3

cyclin dependent kinase 3

Recombinant Human Protein Kinase

HGNC Symbol: CDK3

Synonyms: cell division protein kinase 3

Product No.: 0212-0000-1

Lot: 002

Description: Human CDK3, full length, amino acids M₁-H₃₀₅ (as in NCBI/Protein entry NP_001249.1), N-terminal GST fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: CDK3 Lot 002, was confirmed as CDK3 by specific Western blotting

Theoretical MW_{Fusion Protein}: 67,993 Da

Expression: Baculovirus infected Sf9 cells

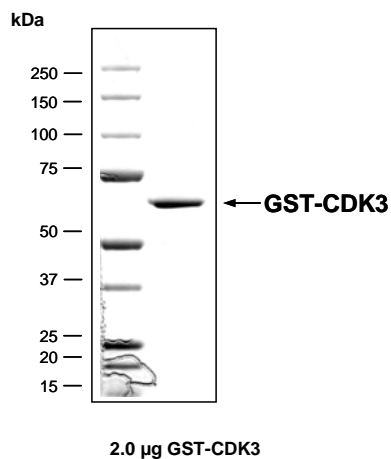
Purification: GST-Affinity Chromatography

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

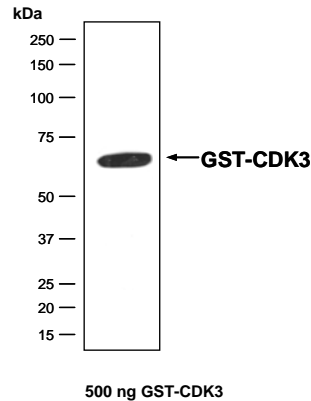
Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!

Protein concentration: 0.183 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

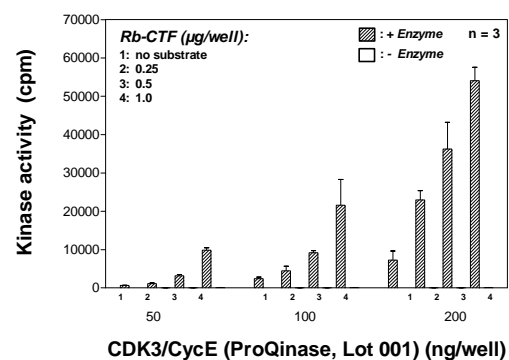
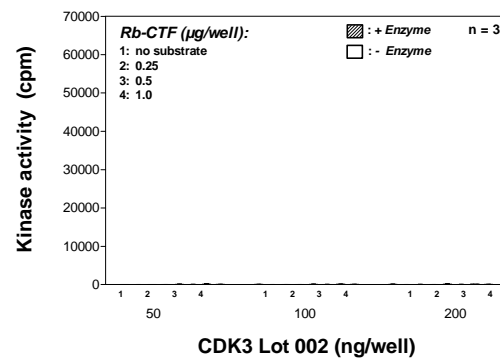
CDK3 Lot 002: Coomassie stain



CDK3 Lot 002: Western Blot Analysis



Kinase activity CDK3 vs active CDK3/CycE:



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): Histone H1
- Recombinant CDK3 or CDK3/CycE (variable)

Assay: ³³PanQinase® Assay

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CDK3 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	PMGHHHHHHG	RRRASVAAGI	240
241	LVPRGSPGLD	GIYARGIQAS	MGARGRQCDG	YLQNSPLAMD	MFQKVEKIGE	GTYGVVYKAK	300
301	NRETGQLVAL	KKIRLDLMEG	VPSTAIRES	LLKELKHPNI	VRLLDVVHNE	RKLYLVFEFL	360
361	SQDLKKYMS	TPGSELPLHL	IKSYLFQLLQ	GVSFCHSHRV	IHRDLKPQNL	LINELGAIKL	420
421	ADFGLARAFG	VPLRITYTHEV	VTLWYRAPEI	LLGSKFYTTA	VDIWSIGCIF	AEMVTRKALF	480
481	PGDSEIDQLF	RIFRMLGTPS	EDTWPQVTQL	PDYKGSFPAK	TRKGLLEIIVP	NLEPEGRDLL	540
	MQLLQYDPSQ	RITAKTALAH	PFSSPEPSP	AARQYVLQRF	RHGKGEFQHT	GARY	

1-218: GST Red: HIS6 Pink: Thrombin blue: CDK3

CDK3 wt ¹ amino acid sequence							
1	MDMFQKVEKI	GEPTYGVVYK	AKNRETGQLV	ALKKIRLDLE	MEGVPSTAIR	EISLLKELKH	60
61	PNIVRLLDVV	HNERKLYLVF	EFLSQDLKKY	MDSTPGSELP	LHLIKSYLFQ	LLQGVSFCHS	120
121	HRVIHRDLKP	QNLLINELGA	IKLADFGLAR	AFGVPLRITYT	HEVVTLWYRA	PEILLGSKFY	180
181	TTAVDIWSIG	CIFAEMVTRK	ALFPGDSEID	QLFRIFRMLG	TPSEDTPWPGV	TQLPDYKGSF	240
241	PKWTRKGLEE	IVPNLEPEGR	DLLMQLLQYD	PSQRITAKTA	LAHPYFSSPE	PSPAARQYVL	300
301	QRFRR						360

blue: CDK3 sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_001249.1