

Certificate of Analysis



CDK16/CycY

cyclin-dependent kinase 16

Recombinant Human Active Protein Kinase

HGNC Symbol: CDK16

Synonyms: PCTAIRE, PCTGAIRE, PCTK1

Product No.: 1117-1116-1

Lot: 001

Description: Coexpression of human CDK16, full length, amino acids M₁-F₄₉₆ (NCBI/Protein entry NP_006192.1), N-terminally fused to GST-HIS₆-3C cleavage site and human CycY, full length, amino acids M₁-S₃₄₁ (NCBI/Protein entry NP_659449.3), N-terminally fused to HIS₆-TEV cleavage site, expressed in Sf9 insect cells

Product identity: CDK16/CycY Lot 001, was confirmed CDK16/CycY by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{GST-CDK16}: 84,221 Da

Theoretical MW_{HIS-CycY}: 41,697 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was activated by coexpression with its physiological cofactor CycY

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: 0.227 µg/µl

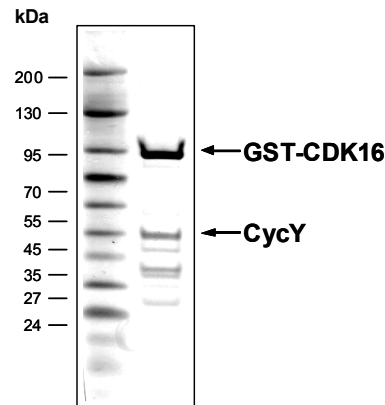
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 7 pmol/µg×min

ATP-K_M: 0.6 µM

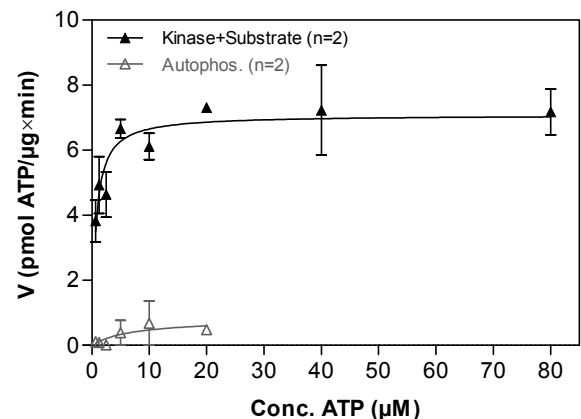
CDK16/CycY Lot 001:
Coomassie stain



2.0 µg CDK16/CycY

CDK16/CycY Lot 001:

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: RBER-GSK3, 200 µg/ml
 - CDK16/CycY: 2.0 µg/ml
- Filter binding assay
 - MSFC membrane (Millipore)

CDK16/CycY

Product No.: 1117-1116-1

CDK16 Recombinant Fusion Protein Amino Acid Sequence								
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60	
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120	
121	DFLSKLP	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180	
181	KRIEAI	PQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHH G	RDS LEVL FQ G	240
241	P LAMV MDRMK	KIKRQ LSMTL	RGGR IDKTN	GAPEQ IGLDE	SGGGG SDPG	EAP TRAAPGE	300	
301	LRSARG PLSS	APEIV HEDLK	MGSDG ESDQA	SATSS DEVQS	PVRVR MRNHP	PRK ISTEDIN	360	
361	KRLSL PADIR	LPEGY LEKLT	LNSPI FDKPL	SRRLR RVSL	EIGFG KLETY	IKL DKLGE	420	
421	YATVY KGKSK	LTDNL VALKE	IRLEH EEGAP	CTAIRE VSL	KDLKH ANIVT	LHDII HTEKS	480	
481	LTLVF EYLDK	DLQYL DDCG	NIINM HNVKL	FLFQL LRGLA	YCHRQ KVLHR	DLKPQ NLLIN	540	
541	ERGEL KLADF	GLARAK SIPT	KTYSN EVVTL	WYRPP DILLG	STDYST QIDM	WGVGC IFYEM	600	
600	ATGRP LPFGS	TVEEQ LHFIF	RILGT PTEET	WPGIL SNEEF	KTYNY PKYRA	EALLS HAPRL	660	
661	DSDGAD LLTK	LLQFE GRNRI	SAEDAM KHPF	FLSLG ERIHK	LPDTS SIFAL	KEIQL QKEAS	720	
721	LRSSM PDSG	RPAFR VVDTE	F				780	

1-218: GST **Red:** HIS6-tag **Green:** 3C **blue:** CDK16

CDK16 wt ¹ amino acid sequence							
1	MDRMK KIKRQ	LSMTL RGGRG	IDKTN GAPEQ	IGLDE SGGGG	GSDPGE APTR	AAPGEL RSAR	60
61	GPLSSA PEIV	HEDLKM GSDG	ESDQA SATSS	DEVQSP VVRV	MRNHPP RKIS	TEDINK RLSL	120
121	PADIRL PEGY	LEKLT LNSPI	FDKPL SRRLR	RVSLSE IGFG	KLETYI KLDK	LGEGTY ATVY	180
181	KGKSK LTDNL	VALKEI RLEH	EEGAP CTAIR	EVSLK LDLKH	ANIVTL LHDII	HTEKSL TLVF	240
241	EYLDK DLKQY	LDDCG NIINM	HNVKL FLFQL	LRGLAY CHRQ	KVLHRD LKPQ	NLLINER GEL	300
301	KLADF GLARA	KS IPT KTYSN	EVVTL WYRPP	DILLG STDYS	TQIDM WGVGC	IFYEMA TGRP	360
361	LFPGS TVEEQ	LHFIF RILGT	PTEET WPGIL	SNEEF KTYNY	PKYRAE ALLS	HAPRLD SDGA	420
421	DLTKL LQFE	GRNRIS AEDA	MKHPF FLSLG	ERIKL PDTT	SIFALKE IQL	QKEASL RSSS	480
481	MPDSG RPAFR	VVDTE					540

blue: CDK16 sequence expressed in fusion protein

¹NCBI/Protein accession number NP_006192.1

Cyclin Y Recombinant Fusion Protein Amino Acid Sequence							
1	MS HHHHH AMT	ENLYFQ GAMV	MGN TTSCVVS	SSPKL RRNAH	SRLESY RPDT	DLSRE DTGCN	60
61	LQHIS DRENI	DDLNM EFNPS	DHPRA STIFL	SKSQ TDVREK	RKSLF FINHHP	PGQIAR KYSS	120
121	CSTIF LDDST	VSQPN LKYTI	KCV ALAIYYH	IKNRD PDGRM	LLDIF DENLH	PLSKSE VPPD	180
181	YDKHN PEQKQ	IYRFV RTLFS	AAQLT AECAI	VTLVY LERLL	TYAEI DICPA	NWKRI VLGAI	240
241	LLASK VWDDQ	AVWNV DYCQI	LKDIT VEDMN	ELERQ FLELL	QFNIN VPSSV	YAKYY FDLRS	300
301	LAEAN NLSFP	LEPLS RERAH	KLEAI SRLCE	DKYK DLRRA	RKRSAS ADNL	TLPRW SPAI	360
361	S						420

Red: HIS6-tag **Green:** TEV **blue:** CycY

Cyclin Y wt ² amino acid sequence (HGNC Symbol: CCNY)							
1	MGN TTSCVVS	SSPKL RRNAH	SRLESY RPDT	DLSRE DTGCN	LQHIS DRENI	DDLNM EFNPS	60
61	DHPRA STIFL	SKSQ TDVREK	RKSLF FINHHP	PGQIAR KYSS	CSTIF LDDST	VSQPN LKYTI	120
121	KCV ALAIYYH	IKNRD PDGRM	LLDIF DENLH	PLSKSE VPPD	YDKHN PEQKQ	IYRFV RTLFS	180
181	AAQLT AECAI	VTLVY LERLL	TYAEI DICPA	NWKRI VLGAI	LLASK VWDDQ	AVWNV DYCQI	240
241	LKDIT VEDMN	ELERQ FLELL	QFNIN VPSSV	YAKYY FDLRS	LAEAN NLSFP	LEPLS RERAH	300
301	KLEAI SRLCE	DKYK DLRRA	RKRSAS ADNL	TLPRW SPAI	S		360

blue: CycY sequence expressed in fusion protein

²NCBI/Protein accession number NP_659449.3 (CCNY)