

Certificate of Analysis

CDK4/cyclin D1, 10 µg

Cyclin-Dependent Kinase 4/cyclin D1, GST-tagged

ThermoFisher
SCIENTIFIC

Part Number: PV4400

Lot Number: 2756110B

Immediate Storage: -80°C

Shipping Conditions: dry ice

5781 Van Allen Way

Carlsbad, CA 92008

Phone: 760.603.7200

www.thermofisher.com

Description:

Recombinant human full-length protein, GST-tagged, expressed in insect cells. No special measures were taken to activate this kinase.

Specific Activity:

200 nmoles of phosphate transferred to RbING peptide substrate (INGSPRTPRRGQNR) per minute per mg of total protein at 30°C. Activity determined at a final protein concentration of 8.33 µg/mL.

Concentration:

0.45 mg/mL total protein as measured using the Bradford protein assay with BSA as a standard.

Calculated **3,660 nM**.

Aliases:

CDK4/CCND1, CMM3, MGC14458

Storage and Handling:

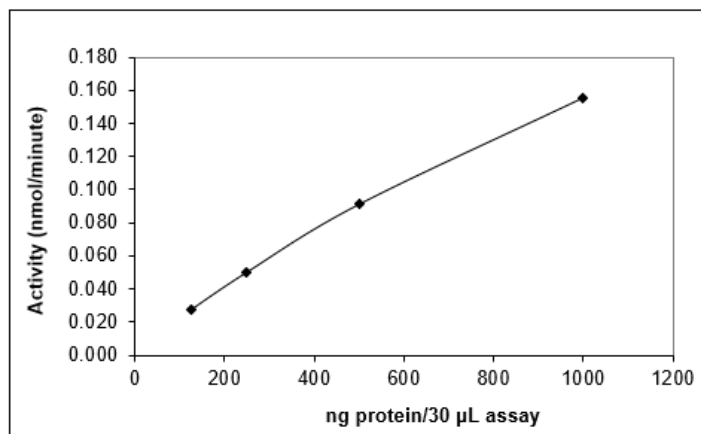
For maximum recovery please spin prior to use. Unless noted below, aliquots of the 5 µg, 10 µg and 20 µg sizes of kinase are not recommended as materials can be used in original packaging until exhausted. For larger sizes, the number of freeze/thaws may be reduced by preparing aliquots, aliquots below 20 µL are not recommended. **Please never store a kinase diluted.** If properly stored at -80°C, this product is guaranteed for 6 months from date of purchase.

Storage Buffer:

50 mM Tris (pH 7.5), 150 mM NaCl, 0.5 mM EDTA, 0.02% Triton® X-100, 2 mM DTT and 50% Glycerol.

QUALITY ASSURANCE

CDK4/cyclin D1 Activity Graph



Dilution Buffer:

20 mM Tris (pH 7.5), 0.02% Triton® X-100, 0.1 mg/mL BSA, 2 mM DTT, 0.5 mM Na₃VO₄ and 10% Glycerol.

Assay Conditions:

CDK4/cyclin D1 was pre-diluted in enzyme dilution buffer and assayed in 25 mM Tris (pH 7.5), 10 mM MgCl₂, 0.5 mM EGTA, 0.5 mM Na₃VO₄, 5 mM β-glycerophosphate, 2.5 mM DTT, 0.01% Triton® X-100, 200 µM ATP, 1000 µg/mL RbING peptide substrate (INGSPRTPRRGQNR) and trace [³²P]-γ-ATP for 10 minutes at 30°C.

Gel Information for CDK4/cyclin D1

Page Description: The SDS-PAGE and/or Native PAGE were run on 4-20% Tris-Glycine Novex™ gels (Catalog #: EC6025BOX).

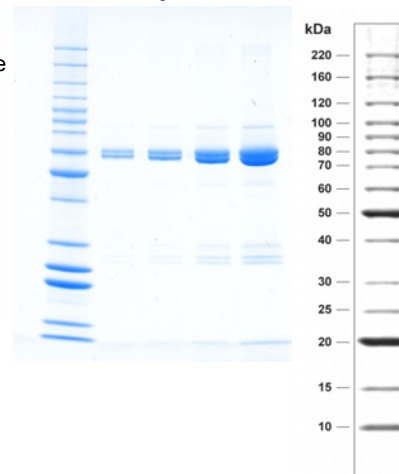
Lane 1: Invitrogen™ BenchMark™ Protein Ladder (Catalog #: 10747-012).

Lane 2: 0.5 µg CDK4/cyclin D1

Lane 3: 1.0 µg CDK4/cyclin D1

Lane 4: 2.5 µg CDK4/cyclin D1

Lane 5: 5.0 µg CDK4/cyclin D1



Purity:

80% as determined by a SDS-PAGE gel stained with SimplyBlue™ SafeStain.

Molecular Weight:

61.8 / 61.2 kDa. Calculated from the protein sequence(s).

Mass Spectrometry:

CDK4/cyclin D1 was subjected to proteolytic digest followed by mass spec analysis. The resulting MS/MS data verified CDK4/cyclin D1 identity by comparison against the amino acid sequence(s) of the recombinant protein.

Part Number: PV4400, Lot Number: 2756110B

GenBank Accession Number: NP_000066

CDK4 Alignment

1	MAPILGYWKI	KGLVQPTRLI	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	GDVKLTQMSA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	GST TAG
1	MAPILGYWKI	KGLVQPTRLI	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	GDVKLTQMSA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	IVGN CDK4/cyclin D1
1											NP_000066
101	DIRYGVSRISA	YSKDFETLKV	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFHLYDALD	VVLYMDPMCL	DAFPKLVCFK	KRIEAIPIQID	KYLKSSKYIA	
101	DIRYGVSRISA	YSKDFETLKV	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFHLYDALD	VVLYMDPMCL	DAFPKLVCFK	KRIEAIPIQID	KYLKSSKYIA	
1											
201	WPLQGWQATF	GGGDHPPKSD	LVPR								
201	WPLQGWQATF	GGGDHPPKSD	LVPRHNTQSL	YKKAGFEGDR	TMATSRYPVP	AEIGVGAYGT	VYKARDPHSG	HFVALKSVRV	PNGGGGGGGL	PISTVREVAL	
1											
224											
301	LRRLAEAFHP	NVVRLLMDVCA	TSRTDREIKV	TLVFEHVDQD	LRTYLDKAPP	PGLPAETIKD	LMRQFLRGLD	FLHANCIVHR	DLKPENILVT	SGGTVKLADF	
60	LRRLAEAFHP	NVVRLLMDVCA	TSRTDREIKV	TLVFEHVDQD	LRTYLDKAPP	PGLPAETIKD	LMRQFLRGLD	FLHANCIVHR	DLKPENILVT	SGGTVKLADF	
224											
401	GLARIYSYQM	ALTPTVVVTLW	YRAPEVLLQS	TYATPVDMMWS	VGCIFAEMFR	RKPLFCGNSE	ADQLGKIFDL	IGLPPEDDW	RDVSLPRGAF	PPRGRPRVQS	
160	GLARIYSYQM	ALTPTVVVTLW	YRAPEVLLQS	TYATPVDMMWS	VGCIFAEMFR	RKPLFCGNSE	ADQLGKIFDL	IGLPPEDDW	RDVSLPRGAF	PPRGRPRVQS	
224											
501	VVPEMEESGA	QLLLEMLTFN	PHKRISAFRA	LQHSYLHKDE	GNPE						
260	VVPEMEESGA	QLLLEMLTFN	PHKRISAFRA	LQHSYLHKDE	GNPE						

GenBank Accession Number: NP_444284

Cyclin D1

1	MAPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EPFNLPYYID	GDVKLTQMSA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	GST TAG
1	MAPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EPFNLPYYID	GDVKLTQMSA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	IVGN CDK4/cyclin D1
1											NP_444284
101	DIRYGVSRIA	YSKDFETLKV	DFLSKLPEML	KMFEDRLCHK	TYLNGDHWTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	KRIEAIPIQID	KYLKSSSKYIA	
101	DIRYGVSRIA	YSKDFETLKV	DFLSKLPEML	KMFEDRLCHK	TYLNGDHWTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	KRIEAIPIQID	KYLKSSSKYIA	
1											
201	WPLQGWQATF	GGGDHPPKSD	LVPR								
1	WPLQGWQATF	GGGDHPPKSD	LVPRHNTQSL	YKKAGTMEHQ	LLCCEVETIR	RAYPDANLLN	DRVLRAMLKA	EETCAPSVSY	FKCVQKEVLP	SMRKIVATWM	
1				-----MEHQ	LLCCEVETIR	RAYPDANLLN	DRVLRAMLKA	EETCAPSVSY	FKCVQKEVLP	SMRKIVATWM	
224											
301	LEVCEEQKCE	EEVFPLAMNY	LDRFLSLPEV	KKSRLQLLGA	TCMFVASKMK	ETIPLTAEKL	CIYTDNSIRP	EELLQMELLL	VNKLKWNLAA	MTPHDFIEHF	
65	LEVCEEQKCE	EEVFPLAMNY	LDRFLSLPEV	KKSRLQLLGA	TCMFVASKMK	ETIPLTAEKL	CIYTDNSIRP	EELLQMELLL	VNKLKWNLAA	MTPHDFIEHF	
224											
401	LSKMPEAEEN	KQIIRKHAQT	FVALCATDVK	FISNPPSMVA	AGSVVAAVQG	LNLRSNNFL	SYRRLTRFLS	RVIKCDPDCL	RACQEQIEAL	LESSLRQAQQ	
165	LSKMPEAEEN	KQIIRKHAQT	FVALCATDVK	FISNPPSMVA	AGSVVAAVQG	LNLRSNNFL	SYRRLTRFLS	RVIKCDPDCL	RACQEQIEAL	LESSLRQAQQ	
224											
501	NMDPKAAEEE	EEEEEEVDLA	CTPTDVRDVD	I.							
265	NMDPKAAEEE	EEEEEEVDLA	CTPTDVRDVD	I.							

* highlighted residues denote differences from the reference protein sequence(s).

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Chevohn Joseph, Director, Quality

Date: 14/Aug/2023

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