Certificate of Analysis
CDK4/cyclin D1 , 10 µg

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

Part Number: PV4400
Lot Number: 2516942K
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:

410 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:

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

Calculated **2,850 nM**.

Aliases:

CDK4/CCND1, CMM3, MGC14458

Storage and Handling:

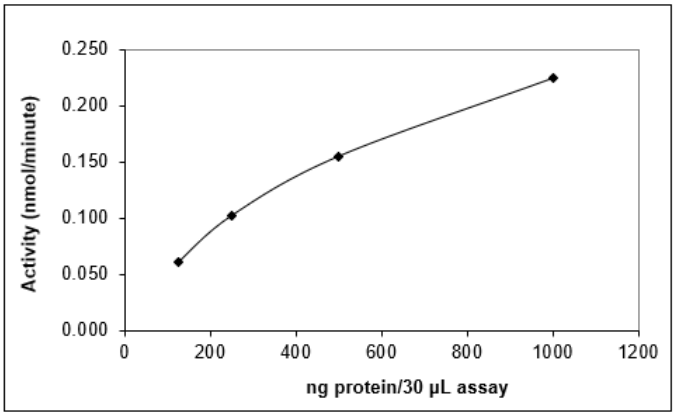
For maximum recovery please spin prior to use. Unless noted below, aliquots of the 5 ug, 10ug and 20ug sizes of kinase are not recommended as materials can be used in original packaging until exhausted. For larger sizes, the number of freeze/thaws may be reduced by preparing aliquots, aliquots below 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



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).

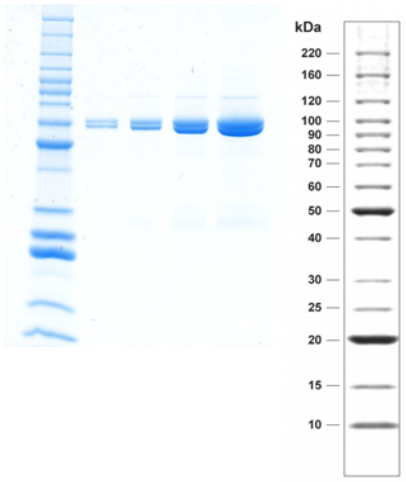
Lane 1: Invitrogen™ BenchMark™ Protein Ladder (Catalog #: 10747-012).

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



Dilution Buffer:

20 mM Tris (pH 7.5), 0.02% Triton® X–100, 0.1 mg/mL BSA, 2 mM DTT 0.5 mM Na_3VO_4 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 $MgCl_2$, 0.5 mM EGTA, 0.5 mM Na_3VO_4, 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 [^{32}P]-γ-ATP for 10 minutes at 30°C.

Purity:

90% 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.

Protein sequence alignment with reference sequence(s)

GenBank Accession Number: NP_000066

CDK4 Alignment

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  1 MAPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL GST TAG
  1 MAPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL IVGN CDK4/cyclin D1
  1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- NP_000066

101 DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA
101 DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA
  1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

201 WPLQGWQATF GGGDHPPKSD LVPR
201 WPLQGWQATF GGGDHPPKSD LVPRHNQTSL YKKAGFEGDR TMATSRYEPV AEIGVGAYGT VYKARDPHSG HFVALKSVRV PNGGGGGGGL PISTVREVAL
  1 ---------- ---------- ---------- ---------- -MATSRYEPV AEIGVGAYGT VYKARDPHSG HFVALKSVRV PNGGGGGGGL PISTVREVAL

224
301 LRRLEAFEHP NVVRLMDVCA TSRTDREIKV TLVFEHVDQD LRTYLDKAPP PGLPAETIKD LMRQFLRGLD FLHANCIVHR DLKPENILVT SGGTVKLADF
 60 LRRLEAFEHP NVVRLMDVCA TSRTDREIKV TLVFEHVDQD LRTYLDKAPP PGLPAETIKD LMRQFLRGLD FLHANCIVHR DLKPENILVT SGGTVKLADF

224
401 GLARIYSYQM ALTPVVVTLW YRAPEVLLQS TYATPVDMWS VGCIFAEMFR RKPLFCGNSE ADQLGKIFDL IGLPPEDDWP RDVSLPRGAF PPRGPRPVQS
160 GLARIYSYQM ALTPVVVTLW YRAPEVLLQS TYATPVDMWS VGCIFAEMFR RKPLFCGNSE ADQLGKIFDL IGLPPEDDWP RDVSLPRGAF PPRGPRPVQS

224
501 VVPEMEESGA QLLLEMLTFN PHKRISAFRA LQHSYLHKDE GNPE.
260 VVPEMEESGA QLLLEMLTFN PHKRISAFRA LQHSYLHKDE GNPE
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GenBank Accession Number: NP_444284

Cyclin D1

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  1 MAPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL GST TAG
  1 MAPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL IVGN CDK4/cyclin D1
  1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- NP_444284

101 DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA
101 DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA
  1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

201 WPLQGWQATF GGGDHPPKSD LVPR
201 WPLQGWQATF GGGDHPPKSD LVPRHNQTSL YKKAGTMEHQ LLCCEVETIR RAYPDANLLN DRVLRAMLKA EETCAPSVSY FKCVQKEVLP SMRKIVATWM
  1 ---------- ---------- ---------- ------MEHQ LLCCEVETIR RAYPDANLLN DRVLRAMLKA EETCAPSVSY FKCVQKEVLP SMRKIVATWM

224
301 LEVCEEQKCE EEVFPLAMNY LDRFLSLEPV KKSRLQLLGA TCMFVASKMK ETIPLTAEKL CIYTDNSIRP EELLQMELLL VNKLKWNLAA MTPHDFIEHF
 65 LEVCEEQKCE EEVFPLAMNY LDRFLSLEPV KKSRLQLLGA TCMFVASKMK ETIPLTAEKL CIYTDNSIRP EELLQMELLL VNKLKWNLAA MTPHDFIEHF

224
401 LSKMPEAEEN KQIIRKHAQT FVALCATDVK FISNPPSMVA AGSVVAAVQG LNLRSPNNFL SYYRLTRFLS RVIKCDPDCL RACQEQIEAL LESSLRQAQQ
165 LSKMPEAEEN KQIIRKHAQT FVALCATDVK FISNPPSMVA AGSVVAAVQG LNLRSPNNFL SYYRLTRFLS RVIKCDPDCL RACQEQIEAL LESSLRQAQQ

224
501 NMDPKAAEEE EEEEEEVDLA CTPTDVRDVD I.
265 NMDPKAAEEE EEEEEEVDLA CTPTDVRDVD I
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* highlighted residues denote differences from the reference protein sequence(s).

Chevohn Joseph, Director, Quality Date: 18/Jul/2022