

Certificate of Analysis

CDK4/cyclin D3, 1 mg

Recombinant human full-length CDK4/cyclin D3

ThermoFisher
SCIENTIFIC

Part Number: A33736

Lot Number: 2516932

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 active CDK4/cyclin D3 were co-expressed in insect cells using each with a N-terminal GST tag.

CDK4 is a member of the cyclin-dependent protein kinase family and is involved in the control of cell proliferation during the G1 phase of cell cycle. CDK4 forms a complex with the D-type cyclins and is inhibited by p16 (cyclin-dependent kinase inhibitor-2). CDK4 can mediate phosphorylation of the C-terminal region of Rb protein leading to an active transcriptional repression of E2F complex. CDC37 and HSP90 can preferentially associate with the fraction of CDK4 not bound to D-type cyclins. SMAD3 is a major physiologic substrate of the G1 cyclin-dependent kinases CDK4 and CDK2.

Accession Numbers:

The gene accession number for the subunits CDK4 and Cyclin D3 are NP_000066.1 and NP_001751.1.

Specific Activity:

6 nmoles of ADP formed per min per mg of total protein using an ADP accumulation assay.

Concentration:

0.1 mg/mL as determined by densitometry of the kinase gel band(s).

Calculated **842 nM**.

Aliases:

CDK4: CMM3; PSK-J3; MGC14458

Cyclin D3: CCND3

Storage and Handling:

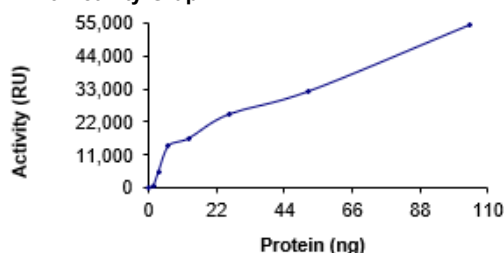
For maximum recovery please spin prior to use. Unless noted below, aliquots of the 5 ug, 10ug and 20ug sizes of kinase are not recommended as materials can be used in original packaging until exhausted. For larger sizes, the number of freeze/thaws may be reduced by preparing aliquots, aliquots below 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-HCl (pH 7.5), 150 mM NaCl, 10 mM Glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF and 25% Glycerol.

QUALITY ASSURANCE

CDK4/cyclin D3 Activity Graph



Dilution Buffer:

40 mM Tris-HCl (pH 7.4), 20 mM MgCl₂, 50 µM DTT and 0.1 mg/mL BSA.

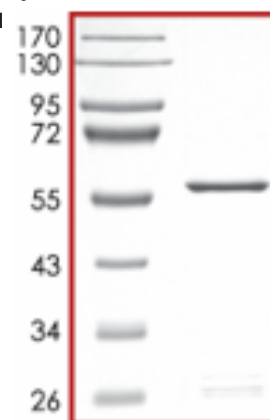
Assay Conditions:

CDK4/cyclin D3 was pre-diluted in enzyme dilution buffer and assayed in 40 mM Tris-HCl (pH 7.4), 20 mM MgCl₂, 50 µM DTT and 0.1 mg/mL BSA with 25 µM ATP in an ADP accumulation assay using 40 µg/mL Rb (773-928) Recombinant Human C-terminal fragment for 40 minutes at room temperature.

Gel Information for CDK4/cyclin D3

Page Description: Run on an SDS-PAGE gel and stained with Coomassie®.

Lane 1: Molecular Weight markers as labeled.



Purity:

> 90% as determined by a Coomassie® blue stained SDS-PAGE gel, excluding endogenous GST.

Molecular Weight:

60.0 / 58.8 kDa. Calculated from the protein sequence(s).

Protein sequence alignment with reference sequence(s)

GenBank Accession Number: NP_000066.1

CDK4

1 MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAISMLEGAVL GST
1 MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAISMLEGAVL Life CDK4/cyclin D3
1 M-----NP_000066.1

101 DIRYGVSRIAYSKDFETLKVDLSKLPMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA
101 DIRYGVSRIAYSKDFETLKVDLSKLPMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA
2 -----

201 WPLQGWQATFGGGDHPPKSD
201 WPLQGWQATFGGGDHPPKSDLVPRGSMATSRYPVAEIGVGAYGTVYKARDPHSGHFVALKSVRVPNGGGGGGLPISTVREALLRRLEAFEHPNVVRL
2 -----ATSRYPVAEIGVGAYGTVYKARDPHSGHFVALKSVRVPNGGGGGGLPISTVREALLRRLEAFEHPNVVRL

220
301 MDVCATSRDREIKVTLVFEHVDQDLRTYLDKAPPPGLPAETIKDLMRQFLRGDLFLHANCIVHRDLKPENILVTSGGTVKLADFLARIYSYQMALTPV
75 MDVCATSRDREIKVTLVFEHVDQDLRTYLDKAPPPGLPAETIKDLMRQFLRGDLFLHANCIVHRDLKPENILVTSGGTVKLADFLARIYSYQMALTPV

220
401 VVTLWYRAPEVLLQSTYATPDMWSVGCIFAEMFRRKPLFCGNSEADQLGKIFDLIGLPPEDDWPRDVS LPRGAFFPRGPRPVQSVVPEMEESGAQLLLE
175 VVTLWYRAPEVLLQSTYATPDMWSVGCIFAEMFRRKPLFCGNSEADQLGKIFDLIGLPPEDDWPRDVS LPRGAFFPRGPRPVQSVVPEMEESGAQLLLE

220
501 MLTFNPHKRISAFRALQHSYLHKDEGNPE
275 MLTFNPHKRISAFRALQHSYLHKDEGNPE

GenBank Accession Number: NP_00175.1

Cyclin D3

1 MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAISMLEGAVL GST
1 MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAISMLEGAVL Lilfe CDK4/cyclin D3
1 M-----NP_00175.1

101 DIRYGVSRIAYSKDFETLKVDLSKLPMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA
101 DIRYGVSRIAYSKDFETLKVDLSKLPMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA
2 -----

201 WPLQGWQATFGGGDHPPKSD
201 WPLQGWQATFGGGDHPPKSDLVPRGSMELLCCEGTRHAPRAGDPRL LGDQRLVLSLLRLEERYVPRASYFCVQREIKPHMRKMLAYWMLVCEEQRCE
2 -----ELLCCEGTRHAPRAGDPRL LGDQRLVLSLLRLEERYVPRASYFCVQREIKPHMRKMLAYWMLVCEEQRCE

220
301 EEVFLAMNYLDRYLSCVPTRKAQLQLLGAVCMLLASKLRETTPLTIEKLCIYTDHAVSPRQLRDWEVLVLGKLKWDLA AVIAHDFLAFILHRLSLPRDR
75 EEVFLAMNYLDRYLSCVPTRKAQLQLLGAVCMLLASKLRETTPLTIEKLCIYTDHAVSPRQLRDWEVLVLGKLKWDLA AVIAHDFLAFILHRLSLPRDR

220
401 QALVKKHAQTFALCATDYTFAMYPSPMIATGSIGAAVQGLGACSMGDELTELLAGITGTEVDCLRACQEQIEAALRESLREASQTSSSPAPKAPRGSS
175 QALVKKHAQTFALCATDYTFAMYPSPMIATGSIGAAVQGLGACSMGDELTELLAGITGTEVDCLRACQEQIEAALRESLREASQTSSSPAPKAPRGSS

220
501 SQGPSQTSTPTDVTAIHL
275 SQGPSQTSTPTDVTAIHL

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

Rachel Smith

Rachel Smith, Manager, QA/QC

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