

Certificate of Analysis

CDK4/cyclin D3, 100 µg

Recombinant human full-length CDK4/cyclin D3



Part Number: A33573

Lot Number: 1954362

Immediate Storage: -80°C

Shipping Conditions: dry ice

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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:

13 nmoles of phosphate transferred to Rb (773-928) Recombinant Human C-terminal fragment per minute per mg of total protein at 30°C.

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:

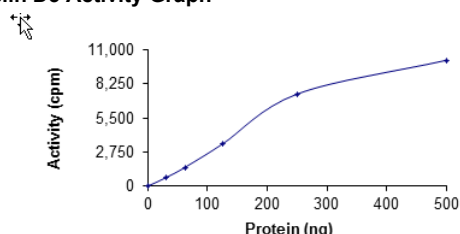
For maximum recovery please spin prior to use. Unless noted below, aliquots of the 5 µg, 10µg and 20µg sizes of kinase are not recommended as materials can be used in original packaging until exhausted. For larger sizes, the number of freeze/thaws may be reduced by preparing aliquots, aliquots below 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, 0.1mM PMSF and 25% Glycerol.

QUALITY ASSURANCE

CDK4/cyclin D3 Activity Graph



Dilution Buffer:

5 mM MOPS (pH 7.2), 2.5 mM β-glycerol-phosphate, 5 mM MgCl₂, 1 mM EGTA, 0.4 mM EDTA and 50 ng/µL BSA.

Assay Conditions:

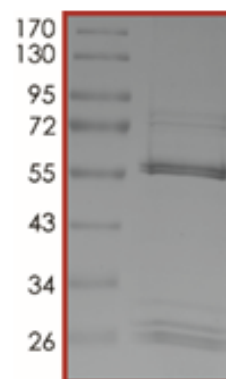
CDK4/cyclin D3 was pre-diluted in enzyme dilution buffer and assayed in 5 mM MOPS (pH 7.2), 2.5 mM β-glycerol-phosphate, 5 mM MgCl₂, 0.4 mM EDTA, 1 mM EGTA, 0.05 mM DTT, with 50 µM ATP, trace [³²P]-γ-ATP and 200 µg/mL Rb (773-928) Recombinant Human C-terminal fragment for 15 minutes at 30°C.

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.

Lane 2: CDK4/cyclin D3



Purity:

> 80% 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 DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA
101 DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA
2 -----

201 WPLQGWQATFGGGDHPPKSD
201 WPLQGWQATFGGGDHPPKSDLVPRGSMATSRYPVAEIGVGAYGTVYKARDPHSGHFVALKSVRVPNGGGGGGLPISTVREALLRRLEAFEHPNVVRL
2 -----ATSRYPVAEIGVGAYGTVYKARDPHSGHFVALKSVRVPNGGGGGGLPISTVREALLRRLEAFEHPNVVRL

220
301 MDVCATSRDREIKVTLVFEHVDQDLRTYLDKAPPPGLPAETIKDLMRQFLRGDLFLHANCIVHRDLKPENILVTSGGTVKLADFLARIYSYQMALTPV
75 MDVCATSRDREIKVTLVFEHVDQDLRTYLDKAPPPGLPAETIKDLMRQFLRGDLFLHANCIVHRDLKPENILVTSGGTVKLADFLARIYSYQMALTPV

220
401 VVTLWYRAPEVLLQSTYATPDMWMSGCIFAEMFRRKPLFCGNSEADQLGKIFDLIGLPPEDDWPRDVS LPRGAFFPRGPRPVQSVVPEMEESGAQLLLE
175 VVTLWYRAPEVLLQSTYATPDMWMSGCIFAEMFRRKPLFCGNSEADQLGKIFDLIGLPPEDDWPRDVS 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 DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA
101 DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA
2 -----

201 WPLQGWQATFGGGDHPPKSD
201 WPLQGWQATFGGGDHPPKSDLVPRGSMELLCCEGTRHAPRAGPDRLLGDQRVLSLLRLEERYVPRASYFCVQREIKPHMRKMLAYWMLVCEEQRCE
2 -----ELLCCEGTRHAPRAGPDRLLGDQRVLSLLRLEERYVPRASYFCVQREIKPHMRKMLAYWMLVCEEQRCE

220
301 EEVFLAMNYLDRYLSCVPTRKAQLQLLGAVMCLLASKLRETTPLTIEKLCIYTDHAVSPRQLRDWEVLVLGKLKWDLAAVIAHDFLAFILHRLSLPRDR
75 EEVFLAMNYLDRYLSCVPTRKAQLQLLGAVMCLLASKLRETTPLTIEKLCIYTDHAVSPRQLRDWEVLVLGKLKWDLAAVIAHDFLAFILHRLSLPRDR

220
401 QALVKKHAQTFALCATDYTFAMYPSPMIATGSIGAAVQGLGACSMGDELTELLAGITGTEVDCLRACQEIEAALRESLREASQTSSSPAPKAPRGSS
175 QALVKKHAQTFALCATDYTFAMYPSPMIATGSIGAAVQGLGACSMGDELTELLAGITGTEVDCLRACQEIEAALRESLREASQTSSSPAPKAPRGSS

220
501 SQGPSQTSTPTDVTAIHL
275 SQGPSQTSTPTDVTAIHL

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

Anita Targosz

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