

# Certificate of Analysis

## CDK4/cyclin D3, 10 µg

Recombinant human full-length CDK4/cyclin D3

**ThermoFisher**  
SCIENTIFIC

**Part Number:** A33515

**Lot Number:** 2034953

**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 CKD4 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:

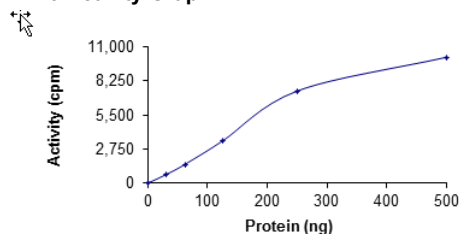
For maximum recovery please spin prior to use. Unless noted below, aliquots of the 5 µg, 10µg and 20µg sizes of kinase are not recommended as materials can be used in original packaging until exhausted. For larger sizes, the number of freeze/thaws may be reduced by preparing aliquots, aliquots below 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 MgCl<sub>2</sub>, 1 mM EGTA, 0.4 mM EDTA and 50 ng/µL BSA.

### Assay Conditions:

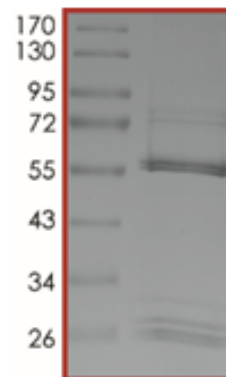
CDK4/cyclin D3 was pre-diluted in enzyme dilution buffer and assayed in 5 mM MOPS (pH 7.2), 2.5 mM β-glycerol-phosphate, 5 mM MgCl<sub>2</sub>, 0.4 mM EDTA, 1 mM EGTA, 0.05 mM DTT, with 50 µM ATP, trace [<sup>32</sup>P]-γ-ATP and 200 µg/mL Rb (773-928) Recombinant Human C-terminal fragment for 15 minutes 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 MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGAVL GST  
1 MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGAVL Life CDK4/cyclin D3  
1 M-----NP\_000066.1

101 DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA  
101 DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA  
2 -----

201 WPLQGWQATFGGGDHPPKSD  
201 WPLQGWQATFGGGDHPPKSDLVPRGSMATSRYPVAEIGVGAYGTVYKARDPHSGHFVALKSVRVPNGGGGGGLPISTVREALLRRLAEFHPNVVRL  
2 -----ATSRYPVAEIGVGAYGTVYKARDPHSGHFVALKSVRVPNGGGGGGLPISTVREALLRRLAEFHPNVVRL

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 MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGAVL GST  
1 MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGAVL Lilfe CDK4/cyclin D3  
1 M-----NP\_00175.1

101 DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA  
101 DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA  
2 -----

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

220  
301 EEVFLAMNYLDRYLSCVPTRKAQLQLLGAVMCLLASKLRETTPLTIEKLCIYTDHAVSPRQLRDWEVLVLGKLKWDLA AVIAHDFLAFILHRLSLPRDR  
75 EEVFLAMNYLDRYLSCVPTRKAQLQLLGAVMCLLASKLRETTPLTIEKLCIYTDHAVSPRQLRDWEVLVLGKLKWDLA AVIAHDFLAFILHRLSLPRDR

220  
401 QALVKKHAQTFALCATDYTFAMYPSPMIATGSIGAAVQGLGACSMGDELTELLAGITGTEVDCLRACQEQIEAALRESLREASQTSSSPAPKAPRGSS  
175 QALVKKHAQTFALCATDYTFAMYPSPMIATGSIGAAVQGLGACSMGDELTELLAGITGTEVDCLRACQEQIEAALRESLREASQTSSSPAPKAPRGSS

220  
501 SQGPSQTSTPTDVTAIHL  
275 SQGPSQTSTPTDVTAIHL

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

Anita Targosz

Anita Targosz, Carlsbad - Engineer, QA

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