

# Certificate of Analysis

## CDK4/cyclin D3, 10 µg

Recombinant human full-length CDK4/cyclin D3

**ThermoFisher**  
SCIENTIFIC

**Part Number:** A33515

**Lot Number:** 3124698

**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:

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

### Concentration:

0.10 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:

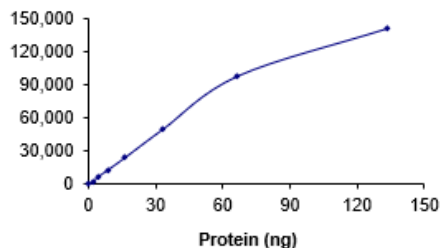
For maximum recovery please spin prior to use. Unless noted below, aliquots of the 5 µg, 10µg and 20µg sizes of kinase are not recommended as materials can be used in original packaging until exhausted. For larger sizes, the number of freeze/thaws may be reduced by preparing aliquots, aliquots below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

## QUALITY ASSURANCE

### CDK4/cyclin D3 Activity Graph



### Dilution Buffer:

40 mM Tris-HCl (pH 7.4), 20 mM MgCl<sub>2</sub>, 50 µM DTT and 0.1 mg/mL BSA.

### Assay Conditions:

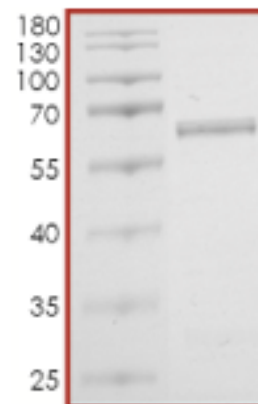
CDK4/cyclin D3 was pre-diluted in enzyme dilution buffer and assayed in 40 mM Tris-HCl (pH 7.4), 20 mM MgCl<sub>2</sub>, 50 µM DTT and 0.1 mg/mL BSA with 25 µM ATP in an ADP accumulation assay using 40 µg/mL Rb (773-928) Recombinant Human C-terminal fragment for 40 minutes at room temperature.

### 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.

**Lane 2:** CDK4/cyclin D3



### 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 MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGAVL GST  
1 MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGAVL Life CDK4/cyclin D3  
1 M-----NP\_000066.1

101 DIRYGVSRIAYSKDFETLKVDLSKLPKMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA  
101 DIRYGVSRIAYSKDFETLKVDLSKLPKMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA  
2 -----

201 WPLQGWQATFGGGDHPKSD  
201 WPLQGWQATFGGGDHPKSDLVPRGSMATSRYPVAEIGVGAYGTVYKARDPHSGHFVALKSVRVPNGGGGGGLPISTVREALLRRLEAFEHPNVVRL  
2 -----ATSRYPVAEIGVGAYGTVYKARDPHSGHFVALKSVRVPNGGGGGGLPISTVREALLRRLEAFEHPNVVRL

220  
301 MDVCATSRDREIKVTLVFEHVDQDLRTYLDKAPPPGLPAETIKDLMRQFLRGLDFLHANCIVHRDLKPENILVTSGGTVKLADFLARIYSYQMALTPV  
75 MDVCATSRDREIKVTLVFEHVDQDLRTYLDKAPPPGLPAETIKDLMRQFLRGLDFLHANCIVHRDLKPENILVTSGGTVKLADFLARIYSYQMALTPV

220  
401 VVTLWYRAPEVLLQSTYATPDMWSVGCIFAEMFRRKPLFCGNSEADQLGKIFDLIGLPPEDDWPRDVS LPRGAFPPRGPVQSVVPEMEESGAQLLLE  
175 VVTLWYRAPEVLLQSTYATPDMWSVGCIFAEMFRRKPLFCGNSEADQLGKIFDLIGLPPEDDWPRDVS LPRGAFPPRGPVQSVVPEMEESGAQLLLE

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 DIRYGVSRIAYSKDFETLKVDLSKLPKMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA  
101 DIRYGVSRIAYSKDFETLKVDLSKLPKMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIA  
2 -----

201 WPLQGWQATFGGGDHPKSD  
201 WPLQGWQATFGGGDHPKSDLVPRGSMELLCCEGTRHAPRAGDPRLLGDQRVLSLLRLEERYVPRASYFQCVQREIKPHMRKMLAYWMLVCEEQRCE  
2 -----ELLCCEGTRHAPRAGDPRLLGDQRVLSLLRLEERYVPRASYFQCVQREIKPHMRKMLAYWMLVCEEQRCE

220  
301 EEVFLAMNYLDRYLSCVPTRKAQLQLLGAVMCLLASKLRETTPLTIEKLCIYTDHAVSPRQLRDWEVLVLGKWKDLAAVIAHDFLAFILHRLSLPRDR  
75 EEVFLAMNYLDRYLSCVPTRKAQLQLLGAVMCLLASKLRETTPLTIEKLCIYTDHAVSPRQLRDWEVLVLGKWKDLAAVIAHDFLAFILHRLSLPRDR

220  
401 QALVKKHAQTFALCATDYTFAMYPSPMIATGSIGAAVQGLGACSMGDELTELLAGITGTEVDCLRACQEQIEAALRESLREASQTSSSPAPKAPRGSS  
175 QALVKKHAQTFALCATDYTFAMYPSPMIATGSIGAAVQGLGACSMGDELTELLAGITGTEVDCLRACQEQIEAALRESLREASQTSSSPAPKAPRGSS

220  
501 SQGPSQTSTPTDVTAIHL  
275 SQGPSQTSTPTDVTAIHL

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



Chevoyn Joseph, Director, Quality

Date: 19/Dec/2024

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