

## CDK4/CyclinD3, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # C31-18G

Lot # W3005-15

### Product Description

Recombinant full-length human CDK4 and CyclinD3 were co-expressed by baculovirus in Sf9 cells using an N-terminal GST tag on both proteins. The gene accession numbers for CDK4 and CyclinD3 are [NM\\_000075](#) and [NM\\_001760](#), respectively.

### Gene Aliases

CDK4: CMM3; PSK-J3; MGC14458

Cyclin D3: CCND3

### Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, and 25% glycerol.

### Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

### Scientific Background

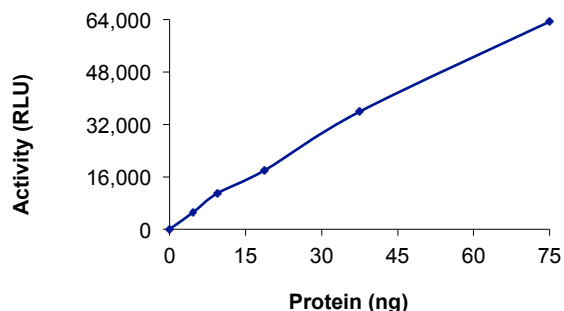
CDK4 is a member of the cyclin-dependent protein kinase family and is involved in the control of cell proliferation during the G1 phase of cell cycle. CDK4 forms a complex with the D-type cyclins and is inhibited by p16 (cyclin-dependent kinase inhibitor-2). CDK4 can mediate phosphorylation of the C-terminal region of Rb protein leading to an active transcriptional repression of E2F complex (1). 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 (2).

### References

1. Harbour, J W. et al: Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. Cell 98: 859-869, 1999.
2. Matsuura, I. et al: Cyclin-dependent kinases regulate the antiproliferative function of Smads. Nature 430: 226-231, 2004.

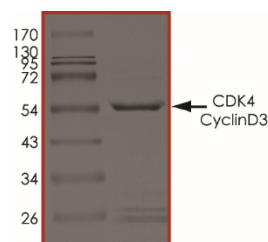
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### Specific Activity



The specific activity of CDK4/CyclinD3 was determined to be equivalent to **6.2 nmol/min/mg** as per activity assay protocol, and was equivalent to **13 nmol/min/mg** as per radiometric assay.

### Purity



The purity of CDK4 /cyclinD3 was determined to be **>75%** by densitometry, approx. MW **58kDa/58kDa**.

## CDK4/CyclinD3, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # C31-18G

Specific Activity 13 nmol/min/mg

Lot # W3005-15

Purity >75%

Concentration 0.1 µg/µl

Stability 1yr at -70°C from date of shipment

Storage & Shipping Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: C31-18G)

Active CDK4/CyclinD3 (0.1µg/µl) diluted with Kinase Dilution Buffer IX (1x) (Catalog #: K29-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of active CDK4/CyclinD3 for optimal results).

### Kinase Assay Buffer III (5x) (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM MgCl<sub>2</sub> and 0.5mg/ml BSA. Add fresh DTT prior to use to a final concentration of 250µM.

### Kinase Dilution Buffer IX (1x) (Catalog #: K29-09)

Kinase Assay Buffer III (Catalog #: K03-09) diluted at a 1:4 ratio (5X dilution) with cold water. Add fresh DTT to the aliquot prior to use to a final concentration of 50µM.

### ADP-Glo™ Kinase Assay Kit (Promega, Cat # V9101)

ATP solution, 10 mM  
ADP solution, 10 mM  
ADP-Glo™ Reagent  
Kinase Detection Reagent

### Substrate (Catalog #: R05-55G)

Rb (773-928) protein substrate prepared in buffer (50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.25mM DTT, 0.1mM PMSF) to a final concentration of 0.2 µg/µl.

## Assay Protocol

The CDK4/CyclinD3 assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the CDK4/CyclinD3 reaction. The ADP- Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

**Step 1.** Thaw the Active CDK4/CyclinD3, Kinase Assay Buffer III (5x), and Substrate on ice. Prepare a 15 µL enzyme dilution at the desired concentration, with Kinase Dilution Buffer IX (1x), in a pre-chilled 96-well plate.

**Step 2.** Prepare a substrate/ATP mixture as follows (25 µM example):

Component	Amount (µL)	Component	Amount (µL)
10mM ATP Solution	1	Substrate at 0.2 mg/mL	80
Kinase Assay Buffer III (5x)	79		

**Step 3.** Transfer the following reaction components prepared in Step 2 to a 384-well opaque plate bringing the reaction volume up to 5µL:

<b>Component 1.</b>	3µl of diluted Active CDK4/CyclinD3 (Catalog # C31-18G).
<b>Component 2.</b>	2µl of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

**Step 4.** Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer IX (1x).

**Step 5.** Incubate at ambient temperature for 40 minutes.

**Step 6.** After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5µl of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.

**Step 7.** Then add 10µl of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.

**Step 8.** Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader (Promega; Cat# E7061).

**Step 9.** Determine the corrected activity (RLU) by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

### Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

### Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected RLU from reaction / [(SA of ADP in RLU/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]

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**FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.**

# MATERIAL SAFETY DATA SHEET

## Article 1 - Product Identification and Use

**Product Name: CDK4/CyclinD3, Active**

**Catalog # C31-18G**

*This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.*

Manufacturer's Name: SignalChem Pharmaceuticals Inc.  
Street Address: 110-13120 Vanier Place  
City, Prov. Postal Code: Richmond, BC, V6V 2J2  
Fax: 604-232-4601  
EMERGENCY PHONE: 604-232-4600

## Article 2 - Hazardous Ingredients

NOT AVAILABLE. We are not aware of any hazards associated with this product or its ingredients, but the chemical, physical, and toxicological properties of this product have not been investigated thoroughly. Observe normal laboratory precautions.

## Article 3 - Physical Data

This product consists of purified protein in Tris-HCl buffer shipped on dry ice. The physical properties of this product have not been investigated thoroughly.

## Article 4 - Fire and Explosion Hazard

NOT APPLICABLE

## Article 5 - Reactivity Data

NOT APPLICABLE

## Article 6 - Toxicologically Data

May be harmful by inhalation, ingestion, or skin absorption. The toxicological properties of this product have not been investigated thoroughly. Exercise due caution.

## Article 7 - Preventative Measures

Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.

\*\*\*\*\*MULTIPLE COMPONENT SPILL OR LEAK PROCEDURES\*\*\*\*\*

- Wear protective equipment.
- Absorb on sand or vermiculite and place in closed containers for disposal.
- Observe all federal, state and local environmental regulations.

## Article 8 - First Aid Measures

- If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
- In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. If a rash or other irritation develops, call a physician.
- If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.
- In case of eye contact, flush with copious amounts of water for at least 15 minutes while separating the eyelids with fingers. Call a physician.

## Article 9 - Preparation

Prepared by: Jun Yan

Phone#: 1-866-954-6273

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.

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