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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
C31-18G-05	5 µg
C31-18G-10	10 µg

CDK4/CyclinD3, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # C31-18G
Lot # Y1092-4

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, 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.

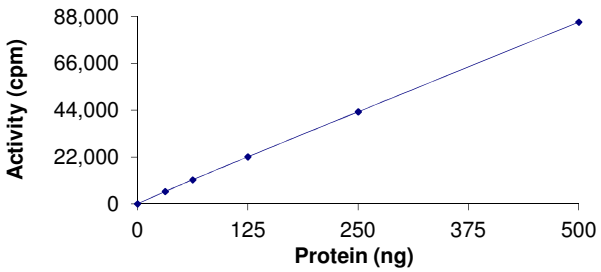
Scientific Background

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

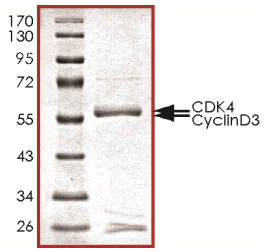
1. Harbour, J W. et al: Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. Cell 98: 859-869, 1999.
2. Matsuura, I. et al: Cyclin-dependent kinases regulate the antiproliferative function of Smads. Nature 430: 226-231, 2004.

Specific Activity



The specific activity of CDK4/CyclinD3 was determined to be **14 nmol/min/mg** as per activity assay protocol using protein substrate Rb (C-Term).

Purity



The purity of CDK4 /cyclinD3 was determined to be **>80%** by densitometry, approx. MW **58kDa/ 58kDa**.

CDK4/CyclinD3, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog #	C31-18G
Specific Activity	14 nmol/min/mg
Lot #	Y1092-4
Purity	>80%
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.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: C31-18G)

Active CDK4/CyclinD3 (0.1µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) 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 Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250µM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [^{33}P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at –20°C.

Substrate (Catalog #: RO5-55G)

Rb (C-Term) 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

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active CDK4 /CyclinD3, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:

- **Component 1.** 10µl of diluted Active CDK4/CyclinD3 (Catalog #C31-18G)
- **Component 2.** 10µl of 0.2mg/ml stock solution of substrate (Catalog #R05-55G)

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5 µl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

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

Calculation of [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [^{33}P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

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

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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