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CDK6/CyclinD3, Active

Full-length recombinant proteins expressed in Sf9 cells

Catalog # C35-10H
Lot # Q008-1

Product Description

Recombinant full-length human CDK6 and CyclinD3 were co-expressed by baculovirus in Sf9 insect cells using an N-terminal His tag on both proteins. The gene accession numbers for CDK6 and CyclinD3 are NM_001259 and NM_001760, respectively.

Gene Aliases

PLSTIRE, MGC59692 /CCND3

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.2mM DTT, 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.

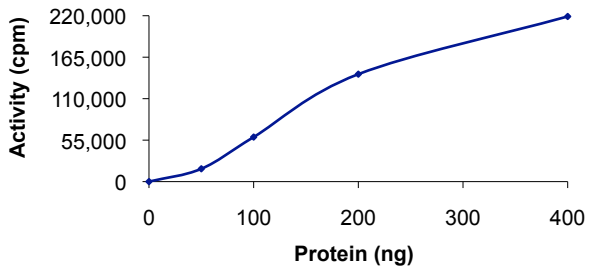
Scientific Background

CDK6 is a member of the cyclin-dependent family of protein kinases that are important regulators of cell cycle progression. CDK6 activity is regulated by the D-type cyclins and members of the INK4 family of CDK inhibitors (1). The CDK6 kinase activity is detected in mid-G1 phase of the cell cycle and is responsible for the phosphorylation and regulation of the activity of tumor suppressor protein Rb. Although CDK6 and CDK4 can both phosphorylate multiple residues in the Rb protein, they do so with different residue selectivities in vitro; CDK6 phosphorylates Thr821 while CDK4 phosphorylates Thr826 on Rb protein (2).

References

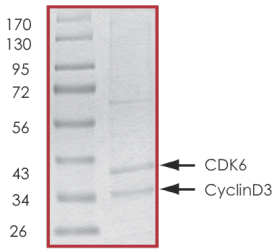
1. Meyerson, M. et al: Identification of G1 kinase activity for cdk6, a novel cyclin D partner. Molec. Cell. Biol. 14: 2077-2086, 1994.
2. Takaki, T. et al: Preferences for phosphorylation sites in the retinoblastoma protein of D-type cyclin-dependent kinases, Cdk4 and Cdk6, in vitro. J Biochem. 2005 Mar;137(3):381-6.

Specific Activity



The specific activity of CDK6/CyclinD3 was determined to be **36 nmol/min/mg** as per activity assay protocol using protein substrate Rb (773-928).

Purity



The purity of CDK6/CyclinD3 was determined to be **>75%** by densitometry, CDK6 approx. MW **40kDa** and CyclinD3 Approx. MW **35kDa**.

CDK6/CyclinD3, Active

Full-length recombinant proteins expressed in Sf9 cells

Catalog Number	C35-10H
Specific Activity	36 nmol /min/mg
Specific Lot Number	Q008-1
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 #: C35-10H)

Active CDK6/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 CDK6/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 final 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 #: R05-55G)

Rb (733-928) protein substrate, 0.2 mg/ml concentration.

Assay Protocol

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

Step 2. Thaw the Active CDK6/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 CDK6/CyclinD3 (Catalog #C35-10H)
- **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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