

CDK9/CyclinK, Active

Full-length recombinant proteins expressed in Sf9 cells

Catalog # C40-10G

Lot # N079-2

Product Description

Recombinant full-length human CDK9 and CyclinK proteins were co-expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession numbers for CDK9 and CyclinK are [NM_001261](#) and [NM_003858](#), respectively.

Gene Aliases

CDC2L4, C-2k, TAK, PITALRE; CCNK, CPR4, MGC9113

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.

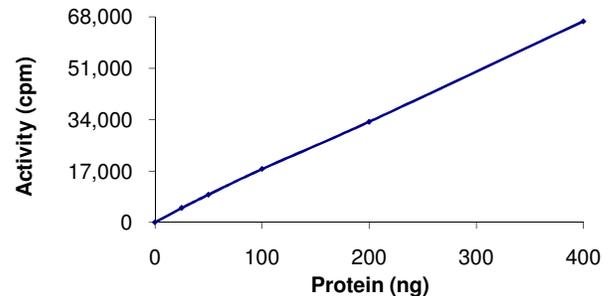
Scientific Background

CDK9/CyclinK is a member of the cyclin-dependent protein kinase (CDK) family. CDK9 is closely related to *cdc28* and *cdc2* and is an important regulator of the cell cycle (1). CDK9 is a component of the multiprotein complex TAK/P-TEFβ. CDK9 can modulate RNA polymerase II-directed transcription by phosphorylating the C-terminal domain of the largest subunit of RNA polymerase II. CDK9 forms a complex with and is regulated by its regulatory subunit cyclin T or cyclin K. CDK9 also interacts with the HIV-1 Tat protein which suggested a possible involvement of this protein in AIDS (2).

References

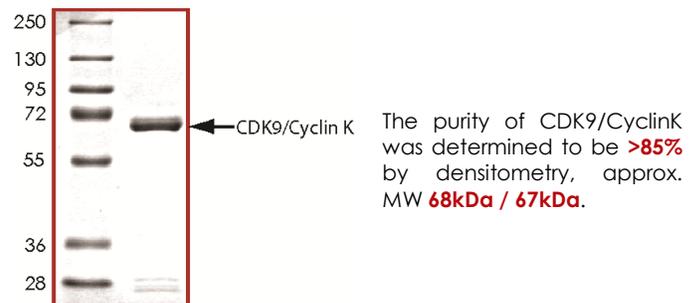
1. Yang, Z. et al: The 7SK small nuclear RNA inhibits the CDK9/cyclin T1 kinase to control transcription. *Nature*, 2001; 414: 317-322.
2. Bullrich, F. et al: Chromosomal mapping of members of the *cdc2* family of protein kinases, *cdk3*, *cdk6*, *P1SSLRE*, and *PITALRE*, and a cdk inhibitor, *p27-Kip1*, to regions involved in human cancer. *Cancer Res.* 1995; 55: 1199-1205.

Specific Activity



The specific activity of CDK9/CyclinK was determined to be **16 nmol /min/mg** as per activity assay protocol.

Purity



CDK9/CyclinK, Active

Full-length recombinant proteins expressed in Sf9 cells

Catalog Number	C40-10G
Specific Activity	16 nmol /min/mg
Specific Lot Number	N079-2
Purity	>85%
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 #: C40-10G)

Active CDK9/CyclinK (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 CDK9/CyclinK 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 MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-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 #: P10-58)

PDKtide synthetic peptide substrate (KTFCGTPEYLAPEVRREP-RILSEEEQEMFRDFDYADWC) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active CDK9/CyclinK, 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 CDK9/CyclinK (Catalog #C40-10G)
 - Component 2.** 5µl of 1 mg/ml stock solution of substrate (Catalog # P10-58)
 - Component 3.** 5µl distilled H₂O (4°C)
- 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 H₂O.
- Step 5.** Initiate the reaction by the addition of 5 µl [³³P]-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 H₂O) 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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³³P]-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 ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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