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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
C36-10H-05	5 µg
C36-10H-10	10 µg

CDK7/CyclinH1/MNAT1, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # C36-10H
Lot # G240-2

Product Description

Recombinant full-length human CDK7, Cyclin H1 and MNATI were co-expressed by baculovirus in Sf9 insect cells using N-terminal His tags. The gene accession number for CDK7, Cyclin H1 and MNAT1 are NM_001799, NM_001239, and NM_002431 respectively.

Gene Aliases

CDK7: CAK1, STK1, CDKN7, p39MO15
Cyclin H1: CCNH, CAK, p34, p37
MNATI: MAT1, RNF66

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.

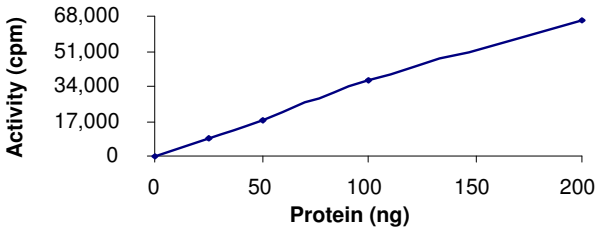
Scientific Background

CDK7 gene is a member of the cyclin-dependent protein kinase family that is important regulators of cell cycle progression (1). CDK7 forms a trimeric complex with cyclin H and MAT1, which functions as a CDK-activating kinase (CAK). CDK7 is an essential component of the transcription factor TFIIH that is involved in transcription initiation and DNA repair. CDK7 is thought to serve as a direct link between the regulation of transcription and the cell cycle (2).

References

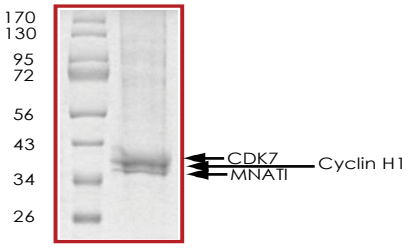
1. Fisher, R. P.;A novel cyclin associates with MO15/CDK7 to form the CDK-activating kinase. *Cell* 78: 713-724, 1994.
2. Larochelle, S. Requirements for Cdk7 in the assembly of Cdk1/cyclin B and activation of Cdk2 revealed by chemical genetics in human cells. *Molec. Cell* 25: 839-850, 2007.

Specific Activity



The specific activity of CDK7/CyclinH1 /MNATI was determined to be **19 nmol /min/mg** as per activity assay protocol.

Purity



The purity of CDK7/CyclinH1/MNAT1 was determined to be **>90%** by densitometry, CDK7 approx. MW **40kDa**, Cyclin H1 approx. MW **39kDa**, and MNAT1 approx. MW **37kDa**

CDK7/CyclinH1/MNAT1, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog #	C36-10H
Specific Activity	19 nmol/min/mg
Lot #	G240-2
Purity	>90%
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 #: C36-10H)

Active CDK7/CyclinH1/MNAT1 (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 CDK7/CyclinH1/MNAT1 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 distilled H_2O.

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 #: M42-51N)

Myelin basic protein (MBP) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

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

Step 2. Thaw the Active CDK7/CyclinH1/MNATI, 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 CDK7/CyclinH1/MNATI (Catalog #C36-10H)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
- **Component 3.** 5μl distilled H_2O (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 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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