3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

CDK7/Cyclin H1/MNAT1, active, His-tagged, human
PRECISIO® Kinase
recombinant, expressed in *Sf*9 cells

Catalog Number **C7745**
Lot Number 081M0796V
Storage Temperature –70 °C

Synonyms:
CDK7: CAK1, STK1, CDKN7, p39MO15
Cyclin H1: CCNH, CAK, p34, p37
MNAT1: MAT1, RNF66

Product Description

CDK7 gene is a member of the cyclin-dependent protein kinase family that is important for regulation 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]

Recombinant full-length human CDK7, Cyclin H1, and MNAT1 were co-expressed by baculovirus in *Sf*9 insect cells using N-terminal His tags. The gene accession numbers for CDK7, Cyclin H1, and MNAT1 are NM 001799, NM 001239, and NM 002431, respectively. It is supplied in 50 mM sodium phosphate, pH 7.0, 300 mM NaCl, 150 mM imidazole, 0.1 mM PMSF, 0.2 mM DTT, and 25% glycerol.

Molecular mass:
CDK7 ~40 kDa
Cyclin H1 ~39 kDa
MNAT1 ~37 kDa

Purity: ≥70% (SDS-PAGE, see Figure 1)

Specific Activity: 17–22 nmole/min/mg (see Figure 2)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Lot Number 081M0796V:
>90% (densitometry)

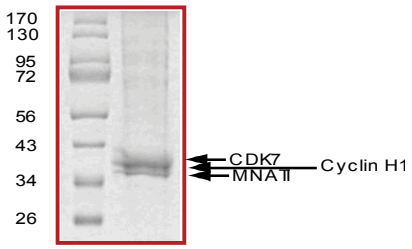
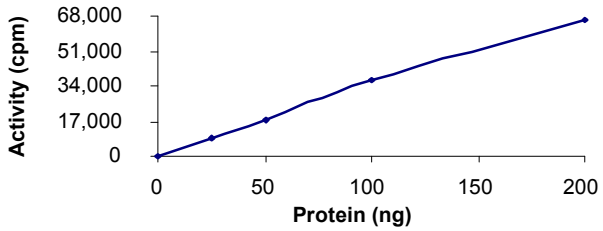


Figure 2.
Specific Activity of Lot Number 081M0796V:
19 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM $MgCl_2$, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with water.

Kinase Solution – Dilute the active CDK7/Cyclin H1/MNAT1 (0.1 μg/μl) with Kinase Dilution Buffer to the desired concentration.
Note: The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active CDK7/Cyclin H1/MNAT1 kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 μl aliquots at –20 °C.

γ-^{32}P-ATP Assay Cocktail (250 μM) – Combine 5.75 ml of Kinase Assay Buffer, 150 μl of 10 mM ATP Stock Solution, 100 μl of γ-^{32}P-ATP (1 mCi/100 μl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the substrate myelin basic protein, MBP, in water at a final concentration of 1 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay
This assay involves the use of the ^{32}P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active CDK7/Cyclin H1/MNAT1, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-^{32}P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 μl:
   10 μl of Kinase Solution
   5 μl of Substrate Solution
   5 μl of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5 μl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 μl of the γ-^{32}P-ATP Assay Cocktail, bringing the final reaction volume to 25 μl. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 μl of the reaction mixture onto an individually precut strip of phosphocellulose P81 paper.
6. Air dry the precut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ-^{32}P-ATP counts introduced into the reaction. Spot 5 μl of the γ-^{32}P-ATP Assay Cocktail on a precut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

Calculations:
1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of 5 μl of γ-}^{32}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

   cpm – value from control (step 7)
   nmole – 1.25 nmole (5 μl of 250 μM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)
Δcpm = cpm of the sample – cpm of the blank (step 3)
25 = total reaction volume
20 = spot volume
T = reaction time (minutes)
E = amount of enzyme (mg)

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).

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