Store at -80°C

#7428

CLK1 Kinase

☑5 µg

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Description: Purified recombinant full length human CLK1 (Met1-Ile484) kinase, supplied as a GST fusion protein.

Background: The cdc2-like kinase (CLK) family contains at least four highly conserved isoforms CLK1, CLK2, CLK3 and CLK4 (1,2). CLKs are dual specific kinases that have been shown to autophosphorylate on serine, threonine and tyrosine residues and phosphorylate exogenous substrates on serine and threonine residues (2). CLK family members can co-exist as full-length catalytically active forms and an alternatively-spliced truncated inactive form (1). A family of highly phosphorylated proteins, so called serine- and arginine-rich (SR) proteins, have been shown to be phosphorylated by CLKs (3-5). SR proteins are splicing factors that regulate the assembly of the spliceosome, a macromolecular complex where RNA splicing occurs in the nucleus, and selection of splice sites. Thus, CLKs may play important roles in regulating RNA splicing.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length human CLK1 (Met1-Ile484) (GenBank Accession No. NM_004071.2) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using GSH-agarose.

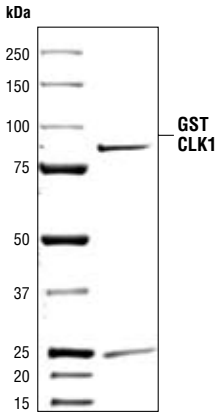

Figure 1. The purity of the GST-CLK1 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-CLK1 fusion protein is 87 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. CLK1 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

(1) Hanes, J. et al. (1994) *J. Mol. Biol.* 244, 665–672.

(2) Nayler, O. et al. (1997) *Biochem J.* 326 (Pt 3), 693–700.

(3) Colwill, K. et al. (1996) *EMBO J.* 15, 265–275.

(4) Prasad, J. and Manley, J.L. (2003) *Mol. Cell Biol.* 23, 4139–4149.

(5) Muraki, M. et al. (2004) *J. Biol. Chem.* 279, 24246–24254.

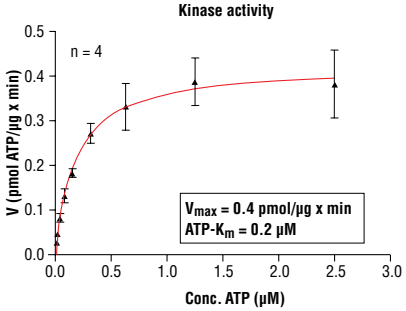

Figure 2. CLK1 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, 2.5 µg/50 µl PEG20,000, ATP variable, Substrate: RS-peptide (RSRSRSRSRSR) 2 µg/50 µL, and recombinant CLK1: 200 ng/50 µl.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione.
Store at –80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

Serine/Threonine Kinase Substrate Screening Kit #7400

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Protocol for CLK1 Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. **Kinase Buffer (5X)**
   300 mM HEPES-NaOH, pH 7.5
   15 mM $MgCl_2$
   15 mM $MnCl_2$
   15 µM Na-orthovanadate
   6 mM DTT
   12.5 µg/50 µl $PEG_{20,000}$
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. RS-peptide (RSRSRSRSRSR) (100 ng/µl)

B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 µM ATP.
2. Dilute [^{32}p] ATP to 0.16 µCi/µl [^{32}p] ATP with 250 µM ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute CLK1 kinase protein to 20 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted CLK1 kinase solution, 10 µl RS-peptide (RSRSRSRSRSR) (100 ng/µL), and 5 µl 0.16 µCi/µl [^{32}p] ATP solution.

Final Assay Conditions
60 mM HEPES-NaOH, pH 7.5
3 mM $MgCl_2$
3 mM $MnCl_2$
3 µM Na-orthovanadate
1.2 mM DTT
50 ng/µl $PEG_{20,000}$
40 ng/µl RS-peptide (RSRSRSRSRSR)

6. After 15 minutes terminate reaction by spotting 20 µl of the reaction mixture onto phosphocellulose P81 paper.
7. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
8. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
9. Count samples in a scintillation counter.

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