Store at -80°C

#7447

CLK2 Kinase

☑5 µg

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Entrez-Gene ID # 1196
Swiss-Prot Acc. # P49760

Description: Purified recombinant human CLK2 (Arg137-Arg498) 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 specificity kinases that autophosphorylate on serine, threonine and tyrosine residues and phosphorylate exogenous substrates on serine and threonine residues (2). CLK family members exist as both a full-length catalytically active form and an alternatively-spliced, inactive truncated form (1). A family of highly phosphorylated proteins, called serine and arginine rich (SR) proteins, are 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. They are also involved in the 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 human CLK2 (Arg137-Arg498) (GenBank Accession No. NM_003993) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

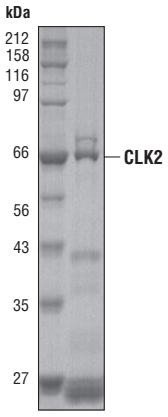

Figure 1. The purity of the GST-CLK2 protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-CLK2 protein is 68 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. CLK2 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, 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.

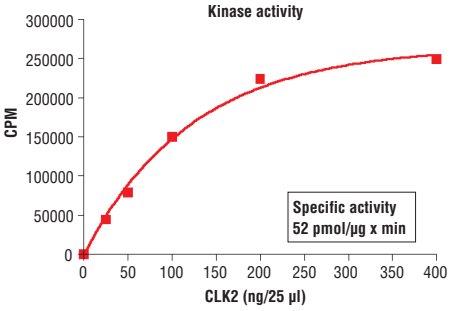

Figure 2. CLK2 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM $MgCl_2$, 0.05 mM DTT, 50 µM ATP, 50 ng/µl BSA, Substrate: S6K peptide 200 ng/µl, and variable amounts of recombinant CLK2.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH7.5; 150 mM NaCl, 0.25 mM DTT, 0.1mM 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:

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

Protocol for CLK2 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)**
   25 mM MOPS, pH 7.2
   12.5 mM β-glycerophosphate
   5 mM EGTA
   2 mM EDTA
   25 mM $MgCl_2$
   0.25 mM DTT
   250 ng/µl BSA
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. S5K peptide (KRRRLASLR, 0.5 µg/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 CLK2 protein (100 ng/µl concentration) to 20 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted CLK2 kinase solution, 10 µl S6K peptide (0.5 µg/µl), and 5 µl 0.16 µCi/µl [32] ATP solution.

Final Assay Conditions
5 mM MOPS, pH 7.2
2.5 mM β-glycerophosphate
1 mM EGTA
5 mM $MgCl_2$
0.05 mM DTT
50 ng/µl BSA
50 µM ATP
200 ng/µl S6K peptide

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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