Store at –80°C

#7728

PLK1 Kinase

☑5 µg

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New 03/07

Description: Purified recombinant full length human PLK1 kinase with an amino-terminal His tag.

Background: At least 4 distinct polo-like kinases exist in mammalian cells: PLK1, PLK2, PLK3 and PLK4/SAK (1). PLK1 apparently plays many roles during mitosis, particularly in regulating mitotic entry and exit. The mitosis promoting factor (MPF), cdc2/cyclin B1, is activated by dephosphorylation of cdc2 (Thr14/Tyr15) by cdc25C. PLK1 phosphorylates cdc25C at Ser198 and cyclin B1 at Ser133 causing translocation of these proteins from the cytoplasm to the nucleus (2-5). PLK1 phosphorylation of Myt1 at Ser426 and Thr495 has been proposed to inactivate Myt1, one of the kinases known to phosphorylate cdc2 at Thr14/Tyr15 (6). Polo-like kinases also phosphorylate the cohesin subunit SCC1, causing cohesin displacement from chromosome arms that allow for proper cohesin localization to centromeres (7). Mitotic exit requires activation of the anaphase promoting complex (APC) (8), a ubiquitin ligase responsible for removal of cohesin at centromeres, and degradation of securin, cyclin A, cyclin B1, Aurora A and cdc20 (9). PLK1 phosphorylation of the APC subunits Apc1, cdc16, and cdc27 has been demonstrated *in vitro* and has been proposed as a mechanism by which mitotic exit is regulated (10,11).

Source/Purification: The kinase protein was produced using a baculovirus expression system using sf9 cells and a recombinant virus encoding full length human PLK1 (Met1-Ser603) (GenBank Accession No. NM_005030) with an amino-terminal His tag. The protein was purified by Immobilized Metal Affinity Chromatography(IMAC).

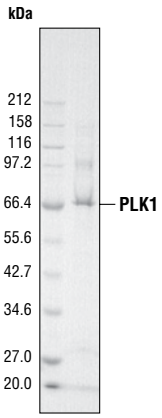

Figure 1. The purity of the PLK1 protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the PLK1 protein is 70 kDa. The purity of the kinase was assessed using SDS-PAGE followed by Coomassie stain [Fig.1]. PLK1 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

(1) Nigg, E.A. (1998) *Curr. Opin. Cell Biol.* 10, 776–783.

(2) Toyoshima-Morimoto, F. et al. (2002) *EMBO Rep.* 3, 341–348.

(3) Toyoshima-Morimoto, F. et al. (2001) *Nature* 410, 215–220.

(4) Peter, M. et al. (2002) *EMBO Rep.* 3, 551–556.

(5) Jackman, M. et al. (2003) *Nat. Cell Biol.* 5, 143–148.

(6) Nakajima, H. et al. (2003) *J. Biol. Chem.* 278, 25277–25280.

(7) Sumara, I. et al. (2002) *Mol. Cell* 9, 515–525.

(8) Hauf, S. et al. (2001) *Science* 293, 1320–1323.

(9) Peters, J.M. (1999) *Exp. Cell Res.* 248, 339–349.

(10) Kraft, C. et al. (2003) *EMBO J.* 22, 6598–6609.

(11) Kotani, S. et al. (1998) *Mol. Cell* 1, 371–380.

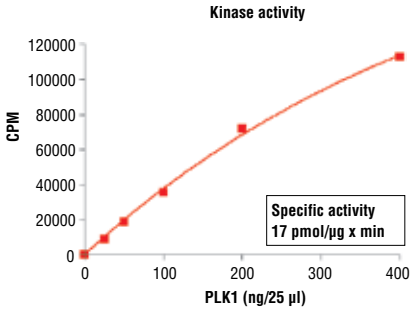

Figure 2. PLK1 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM $MgCl_2$, 0.05 mM DTT, 50 µM ATP, Substrate: Dephospho-Casein 200 ng/µL, and recombinant PLK1: variable.

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:
Tyrosine Kinase Substrate Screening Kit #7450

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Protocol for PLK1 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 (10X)**
   50 mM MOPS, pH 7.2
   25 mM β-glycerophosphate
   10 mM EGTA
   4 mM EDTA
   50 mM $MgC1_2$
   0.5 mM DTT
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. Dephospho-Casein (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 PLK1 protein to 40 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted PLK1 kinase solution, 10 µl Dephospho (0.5 µg/µl), and 5 µl 0.16 µCi/µl [^{32}p] ATP solution.

Final Assay Conditions
5 mM MOPS, pH 7.2
2.5 mM β-glycerophosphate
1 mM EGTA
4 mM $MgCl_2$
0.05 mM DTT
200 ng/µL Dephospho-Casein

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.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: drugdiscovery@cellsignal.com.