Chk1 Kinase

#7359 Store at –80°C

☑5 µg

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: Purified recombinant full-length human Chk1 (Met1-Thr476) kinase, supplied as a GST fusion protein.

Background: Chk1 kinase acts downstream of ATM/ATR kinase to play an important role in DNA damage checkpoint control, embryonic development and tumor suppression (1). Activation of Chk1 involves phosphorylation of Ser317 and Ser345 and occurs in response to blocked DNA replication and certain forms of genotoxic stress (2). Chk1 is also phosphorylated at Ser280 and Ser296 following DNA damage. Activated Chk1 can inactivate cdc25C via phosphorylation at Ser216, blocking the activation of cdc2 and transition into mitosis (3). Chk1 can also phosphorylate p53 at Ser20 *in vitro* (4).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length human Chk1 (Met1-Thr476) (GenBank Accession No. NM_001274) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

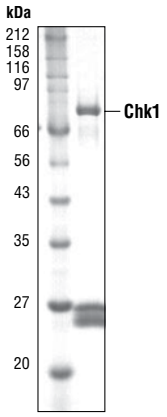

Figure 1. The purity of the GST-Chk1 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-Chk1 fusion protein is 82 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. Chk1 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

(1) Martinho, R.G. et al. (1998) *EMBO J.* 17, 7239–7249.

(2) Zhao, H. et al. (2001) *Mol. Cell. Biol.* 21, 4129–4139.

(3) Zeng, Y. et al. (1998) *Nature* 395, 507–510.

(4) Shieh, S. et al. (2000) *Genes Dev.* 14, 289–300.

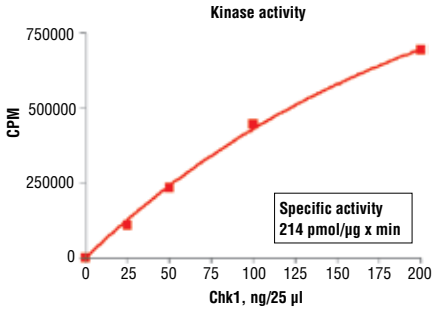

Figure 2. Chk1 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM $MgCl_2$, 0.05 mM DTT, 50 µM ATP, Substrate: CHKtide 400 ng/µL, and variable amounts of Chk1 kinase.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH7.5; 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:

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Serine/Threonine Kinase Substrate Screening Kit #7400

Protocol for Chk1 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 $MgCl_2$
   0.5 mM DTT
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. CHKtide (KKKVSRSGLYRSPSMPENLNRPR) (1 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 Chk1 kinase 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 Chk1 kinase solution, 10 µl CHKtide (1.0 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
0.4 mM EDTA
4 mM $MgCl_2$
0.05 mM DTT
400 ng/µL CHKtide

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.