CHK1 (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPC47

Lot No. B128-2
5 µg 0.1 µg/µl

Background:

CHK1 is a 56 kd serine/threonine protein kinase that was originally identified in fission yeast to play a role in activation of the DNA damage checkpoint in the G2 phase of the cell cycle (1). CHK1 appears to function downstream of several of the known fission yeast checkpoint gene products, including that encoded by rad3+, a gene with sequence similarity to the ATM gene mutated in patients with ataxia telangiectasia (2).

Product Description:

Recombinant full-length human CHK1 was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_001274.

Gene Aliases:

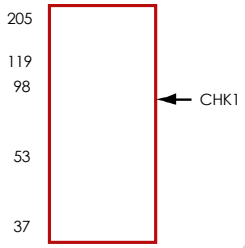
CHEK1

Formulation:

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Purity & Molecular Weight:

The purity was determined to be >90% by densitometry. Approx. MW 82kDa.



Storage:

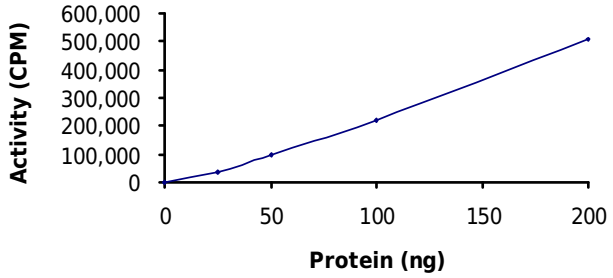
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

Unopened vial at –70 °C, 1 year from date of shipment.

Specific Activity:

The specific activity was determined to be 129 nmol/min/mg as per Activity Assay Protocol.



Activity Assay Protocol:

Assay activity of the kinase in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 10 µL of 1 mg/ml the Substrate Solution, 10 µL of diluted kinase and 5 µL of 250 µM ATP solution containing [gamma ^{32}P] ATP (0.167 µCi/µL). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 µL of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

Substrate Solution:

CHKtide synthetic peptide substrate (KKKVSRSGLYRSPSMPENLNRPR) diluted in distilled H_2O to a final concentration of 1 mg/ml.

5 X Kinase Assay Buffer:

25mM MOPS, 12.5mM β-glycerol-phosphate, 25mM $MgCl_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

1.Walworth, N. et al: Fission yeast CHK1 protein kinase links the rad checkpoint pathway to cdc2. Nature. 1993 May 27;363(6427):368-71.

2.Walworth, NC. et al: Rad-dependent response of the CHK1-encoded protein kinase at the DNA damage checkpoint. Science. 1996 Jan 19;271(5247):353-6.

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