CHK2 (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPC48

Lot No. A288-1
5 µg 0.1 µg/µl

Background:

CHK2 is rapidly phosphorylated and activated in response to replication blocks and DNA damage; the response to DNA damage occurs in an ataxia telangiectasia mutated (ATM)-dependent manner (1). Expression of wild-type Chk2 leads to increased p53 stabilization after DNA damage, whereas expression of a dominant-negative Chk2 mutant abrogated both phosphorylation of p53 on Ser-20 and p53 stabilization (2).

Product Description:

Recombinant full-length human CHK2 was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_007194.

Gene Aliases:

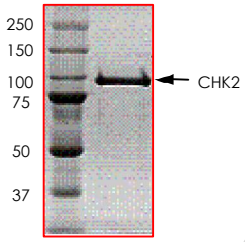
RP11-436C9.1, CDS1, CHEK2, HuCds1, LFS2, PP1425, RAD53

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 88kDa.



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 808 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, 5 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.Matsuoka, S. et al: Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science. 1998 Dec 4;282(5395):1893-7.

2.Chehab NH. et al: Chk2/hCds1 functions as a DNA damage checkpoint in G(1) by stabilizing p53. Genes Dev. 2000 Feb 1;14(3):278-88.

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