

## CHK2, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # C48-10G

Lot # A288-1

### Product Description

Recombinant full-length human CHK2 was expressed by baculovirus in Sf9 insect cells using an 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.

### Storage and Stability

Store product at  $-70^{\circ}\text{C}$ . 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.

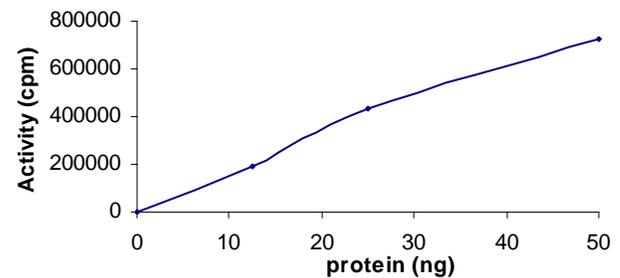
### Scientific Background

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).

### References

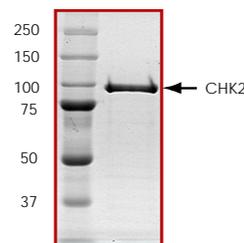
1. Matsuoka, S. et al: Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. *Science*. 1998 Dec 4;282(5395):1893-7.
2. Chehab NH. et al: Chk2/hCds1 functions as a DNA damage checkpoint in G(1) by stabilizing p53. *Genes Dev*. 2000 Feb 1;14(3):278-88.

### Specific Activity



The specific activity of CHK2 was determined to be **808 nmol /min/mg** as per activity assay protocol.

### Purity



The purity was determined to be **>90%** by densitometry. Approx. MW **88kDa**.

## CHK2, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number C48-10G

Specific Activity 808 nmol/min/mg

Specific Lot Number A288-1

Purity	>90%
Concentration	0.1μg/μl
Stability	1yr At $-70^{\circ}\text{C}$ from date of shipment
Storage & Shipping	Store product at $-70^{\circ}\text{C}$ . 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. Product shipped on dry ice.

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# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: C48-10G)

Active CHK2 (0.1µg/µl) diluted with Kinase Dilution Buffer I (Catalog #: K21-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active CHK2 for optimal results).

### Kinase Dilution Buffer I (Catalog #: K21-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with distilled H<sub>2</sub>O.

### Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>32</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>32</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [<sup>32</sup>P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

### 10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at -20°C.

### Substrate (Catalog #: C10-58)

CHKtide synthetic peptide substrate (KKKVSRSGLYRSPSPENLNRPR) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1. Thaw [<sup>32</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active CHK2, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
  - Component 1. 10µl of diluted Active CHK2 (Catalog #C48-10G)
  - Component 2. 10µl of 1mg/ml stock solution of substrate (Catalog #C10-58)
- Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 5. Initiate the reaction by the addition of 5µl [<sup>32</sup>P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H<sub>2</sub>O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

### Calculation of [<sup>32</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [<sup>32</sup>P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

### Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of <sup>32</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

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