Recombinant Human Active Chk2

Certificate of Analysis

Catalog Number: 1358-KS
Lot Number: 1475428

Specifications and Use

Source	♦ Recombinant full-length human Chk2 was expressed by baculovirus in *Sf* 9 insect cells using an N-terminal GST tag. The gene accession number is NM_007194.
Molecular Mass	♦ The approximate molecular weight is 88 kDa (see Figure 1 below).
Purity	♦ The purity was determined to be > 90% by densitometry (see Figure 1 below).
Formulation	♦ Supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 10 mM GSH, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol.
Size	♦ 10 µg.
Concentration	♦ 0.1 µg/µL.
Activity	♦ The specific activity of Chk2 was determined to be 660 nmol/min/mg using a synthetic peptide substrate (KKKVSRSGLYRSPSMPENLNRPR) (see activity assay protocol).
Storage	♦ This product is stable at ≤ -70 °C for up to 1 year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. ♦ **Avoid repeated freeze-thaw cycles.**

Chk2

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 Serine 20 and p53 stabilization (2).

References

1. Matsuoka, S. *et al*. (1998) Science **282**:1893.
2. Chehab, N.H. *et al*. (2000) Genes Dev. **14**:278.

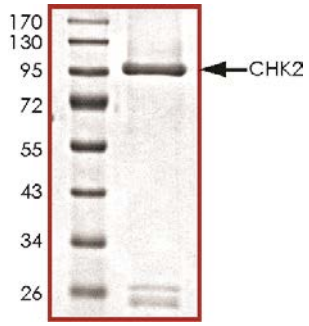

Figure 1: SDS-PAGE and coomassie stain. The approximate molecular weight is 88 kDa and the purity is > 90%.

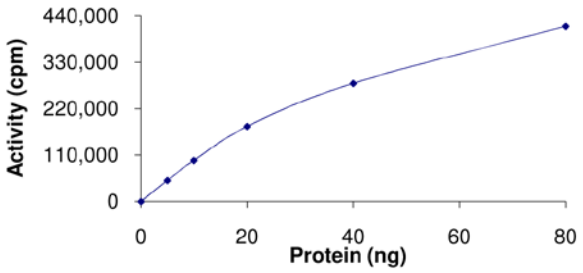

Figure 2: Enzymatic assay results. The specific activity of Chk2 was determined to be 660 nmol/min/mg as per activity assay protocol (on reverse).

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Activity Assay Protocol

Solutions Required

- **Active Kinase** - Active Chk2 (0.1 μg/μL) diluted with Kinase Dilution Buffer and assayed as outlined in Figure 2.
  Note: *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*
- **Kinase Assay Buffer, pH 7.2** - 25 mM MOPS, 12.5 mM β-glycerolphosphate, 25 mM $MgCl_2$, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer prior to use.
- **Kinase Dilution Buffer** - Kinase Assay Buffer diluted at a 1:4 ratio (5-fold dilution) with distilled or deionized water.
- **10 mM ATP Stock Solution** - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer.
- **[^{33}P]-ATP Assay Cocktail** - Prepare 250 μM [^{33}P]-ATP Assay Cocktail in a designated radioactive work area by combining 150 μL of 10 mM ATP Stock Solution, 100 μL of [^{33}P]-ATP (1 mCi/100 μL), and 5.75 mL of Kinase Assay Buffer.
- **Substrate** - Chk-tide synthetic peptide substrate (KKKVSRSGLYRSPSMPENLNRPR) diluted in distilled or deionized water to a final concentration of 1 mg/mL.

Assay Procedure

1. Thaw the [^{33}P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area.
2. Thaw the Active Chk2, Kinase Assay Buffer, Substrate, and Kinase Dilution Buffer on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 μL.

Reaction Component	Amount
Diluted Active Chk2	10 μL
Substrate (1 mg/mL Stock Solution)	5 μL
Distilled H_2O (2-8 °C)	5 μL

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 or deionized water.
5. Initiate the reaction by the addition of 5 μL [^{33}P]-ATP Assay Cocktail, bringing the final volume up to 25 μL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
6. After the 15 minute incubation period, terminate the reaction by spotting 20 μL of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10 mL of phosphoric acid and make a 1 liter solution with distilled or deionized water) with constant gentle stirring. It is recommended that the strips be washed a total of three times for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
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 [^{33}P]-ATP Specific Activity (SA) (cpm/pmol)
Specific Activity (SA) = cpm for 5 μL [^{33}P]-ATP/pmoles of ATP (in 5 μL of a 250 μM ATP stock solution, *i.e*. 1250 pmoles)

Calculation of Kinase Specific Activity (SA) (pmol/minutes/μg or nmol/minutes/mg)
Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in μg or mg)] x [(Reaction volume) / (Spot Volume)]

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