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R&D SYSTEMS

Recombinant Human Active Chk2, CF

Catalog Number: 1358-KS-010
Lot Number: 1784519

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.
Purity	The purity was determined to be > 90% by densitometry.
Formulation	Supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM Glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of Chk2 was determined to be 695 nmol/min/mg using a synthetic peptide substrate (KKKVSRSGLYRSPSMPENLNRPR).
Storage	This product is stable at ≤ -70 °C for up to one 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.**

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. 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.

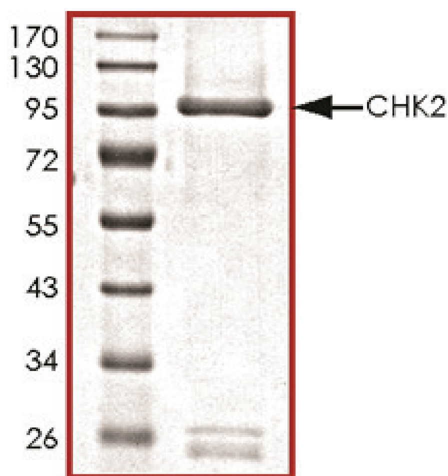

Figure 1: The purity of Chk2 was determined to be > 90% by densitometry. Approximately molecular weight is 88 kDa.

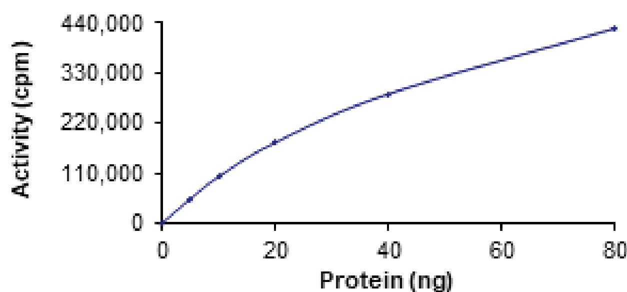

Figure 2: Enzymatic assay results. The specific activity of Chk2 was determined to be 695 nmol/min/mg as per Activity Assay Protocol.

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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 Sample Activity Plot.
Note: *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*

Kinase Dilution Buffer I - Kinase Assay Buffer I diluted at a 1:4 ratio (5X dilution) with distilled water.

Kinase Assay Buffer I - 25 mM MOPS pH 7.2, 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 I prior to use.

[^{32}P]-ATP Assay Cocktail - Prepare 250 µM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 µL of 10 mM ATP Stock Solution, 100 µL [^{33}P]-ATP, 5.75 mL of Kinase Assay Buffer II. Store 1 mL aliquots at ≤ -20 °C.

10 mM ATP Stock Solution - Prepare ATP stock solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer II. Store 200 µL aliquots at ≤ -20 °C.

Substrate - Chktide synthetic peptide substrate (KKKVSRSGLYRSPSMPENLNRPR) diluted in distilled water to a final concentration of 1 mg/mL.

ASSAY PROCEDURE

1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
2. Thaw the Active Chk2, Kinase Assay Buffer, Substrate and Enzyme 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
Stock solution of Substrate at 1 mg/mL	5 µL
Distilled water (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 water.
5. Initiate the reaction by the addition of 5 µL [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25 µL and 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 1L solution with distilled water) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals 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)

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

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol) * (Reaction time in min) * (Enzyme amount in µg or mg)] * [(Reaction Volume) / (Spot Volume)]