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Product Specification

CHK1, active
(Full-length recombinant protein expressed in Sf 9 cells)
Catalog #: 7735
Lot #: _____
Aliquot size: 5 μg protein in 50 μl
Specific activity: 369 nmol/min/mg

Quality Control Analysis
Activity assessment
CHK1 protein (~100 ng/μl concentration) was diluted to 20ng/μl in assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10μl diluted proteins were used to phosphorylate the CHKtide (KKKVSRSGLYRSPSMPENLNRPR) in the assay condition:

10 μl diluted CHK1 protein
5 μl CHKtide (1 mg/ml stock)
5 μl water
5 μl [^{32}P] ATP mixture (250 μM ATP, 0.16 μCi/μl in 4x assay dilution buffer)

The various reaction components, except [^{32}P] ATP, were incubated at 30^{0}C and the reaction started by the addition of [^{32}P] ATP. After 15 minutes, the reaction was terminated by spotting 20 μl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

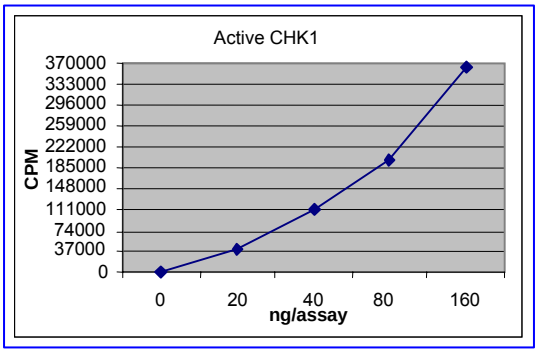

Fig. 1 CHK1 activity assay

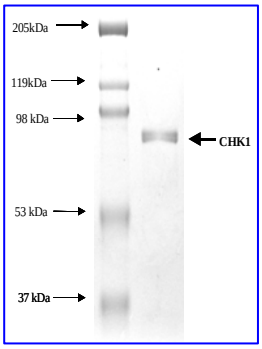

Fig. 2 CHK1 protein gel

Purity assessment
1 μg of CHK1 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the CHK1 band product, and the band was at ~82 kDa (Fig. 2)

Product Description
Recombinant full length human CHK1 containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.
The gene accession number is NM_001274.
This material is sold for research purposes only.

Specific Activity
369 nmol phosphate incorporated into CHKtide per minute per mg protein at 30ºC for 15 minutes using a final concentration of 50 μM ATP (0.83 μCi/assay).

Formulation
Recombinant protein in storage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 30% glycerol.).

Storage and Stability
Store product frozen at or below -70^{0}C. Stable for 1 year at -70^{0}C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific 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). *In vitro,* CHK1 binds to and phosphorylates the dual-specificity protein phosphatases Cdc25A, Cdc25B, and Cdc25C, which control cell cycle transitions by dephosphorylating cyclin-dependent kinases (3). CHK1 phosphorylates Cdc25C on serine-216 which creates a binding site for 14-3-3 protein and inhibits function of the phosphatase. Thus, in response to DNA damage, CHK1 phosphorylates and inhibits Cdc25C, thereby preventing the activation of the Cdc2-cyclin B complex and mitotic entry.

References
1. Walworth N, Davey S, Beach D. Fission yeast CHK1 protein kinase links the rad checkpoint pathway to cdc2. Nature. 1993 May 27;363(6427):368-71.
2. Walworth NC, Bernards R. rad-dependent response of the CHK1-encoded protein kinase at the DNA damage checkpoint. Science. 1996 Jan 19;271(5247):353-6.
3. Sanchez Y, Wong C, Thoma RS, Richman R, Wu Z, Piwnica-Worms H, Elledge SJ. Conservation of the CHK1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. Science. 1997 Sep 5;277(5331):1497-501.