

3050 Spruce Street Saint Louis, Missouri 63103 USA Telephone 800-325-5832 • (314) 771-5765 Fax (314) 286-7828 email: techserv@sial.com sigma-aldrich.com

Checkpoint Kinase 2 (Chk2), Active Human, recombinant, expressed in *E. coli* 

Product Number **C 8866** Storage Temperature: -70 °C

Synonym: S. pombe homolog, Chek2

# **Product Description**

Chk2 is the mammalian homolog of the Saccharomyces cerevisiae Rad53 and Schizosaccharomyces pombe Cds1 protein kinase required for DNA damage and replication checkpoints. Chk2 is rapidly activated, by phosphorylation, in response to replication blocks and DNA damage; the response to DNA damage occurs in an ataxia telangiectasia-mutated (ATM)-dependent manner.<sup>1</sup> In vitro, Chk2 phosphorylates p53 on Ser20 and dissociated preformed complexes of p53 with Mdm2, a protein that targets p53 for degradation.<sup>2</sup> In vivo, ectopic 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 Ser20 and p53 stabilization. Thus, in response to DNA damage, Chk2 stabilizes the p53 tumor suppressor protein leading to cell cycle arrest in G1. ATM directly phosphorylates Chk2 in response to ionizing radiation. The phosphorylation occurs in the Ser-Gln/Thr-Gln (SQ/TQ) cluster domain (SCD), which contains seven SQ/TQ motifs, and Thr<sup>68</sup> is the major in vitro phosphorylation site by ATM.<sup>3</sup>

The product is active recombinant, full-length human Checkpoint Kinase 2 (Chk2) containing an N-terminal GST tag. It is supplied at a concentration of approximately 100  $\mu$ g/mL in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1mM PMSF, and 25% glycerol.

<u>Purity</u>:  $\geq$  85% (SDS-PAGE)

Molecular weight: ~88 kDa

**ProductInformation** 

<u>Specific Activity</u>:  $\geq$  50 units/mg protein (Bradford). Please refer to the Certificate of Analysis for the lot-specific activity.

<u>Unit Definition</u>: One unit will incorporate one nanomole of phosphate into the CHKtide substrate per minute at 30 °C at pH 7.2 using a final concentration of 50  $\mu$ M [<sup>32</sup>P] ATP.

## **Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

## **Preparation instructions**

For maximum product recovery, after thawing, centrifuge the vial before removing the cap

#### Storage/Stability

Stable for at least 12 months when stored as undiluted stock at -70 °C. After initial thawing, store in smaller, working aliquots at -70 °C. Use the working aliquots immediately upon thawing. Avoid repeated freeze-thaw cycles to prevent denaturing of the protein. Do not store in a frost-free freezer.

#### References

- Matsuoka S., et al., Linkage of ATM to cell cycle regulation by the Chk2 protein kinase., Science, 282, 1893-1897 (1998).
- Chehab N.H., et al., Chk2/hCds1 functions as a DNA damage checkpoint in G(1) by stabilizing p53., Genes Dev., 14, 278-288 (2000).
- Matsuoka S., et al., *Ataxia telangiectasia*-mutated phosphorylates Chk2 *in vivo and in vitro*. Proc. Nat. Acad. Sci., U.S.A., **97**, 10389-10394 (2000).

AH,PHC 05-05-1

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.