



Chk1 Positive Control

Product Data Sheet

For Research Use Only, Not for use in diagnostic procedures

Chk1 Positive Control

(Human, full length, recombinant enzyme expressed in Sf9)

Cat# CY-E1162-1

Lot No.

For 200 Assays

2 units (0.005 units / μ L)

Product Description: Human full length Chk1, containing a N-terminal GST tag and a C-terminal His tag, expressed in recombinant baculovirus infected sf9 cells. Purified by sequentially using GSH agarose and Ni-NTA agarose chromatography. The Chk1 Positive control is designed to use for Checkpoint Kinase Assay/Inhibitor Screening Kit -1 [Cat# CY-1162]. The Chk1 Positive Control should be added to the well at 10 m units/well. For instance, diluted positive control 1:5, use 10 μ L for 1 assay. Unused Chk1 Positive control should be stored at -70°C.

Product Size: Full length Chk1: 2 units/400 μ L

Formulation: The Chk1 Positive Control is supplied frozen in a buffer containing 20mM HEPES-KOH (pH 7.5), 1 % BSA, 1mM EDTA, 2 mM DTT, 50mM NaCl, 0.03 % Brij35 and 50% glycerol.

Source: Human full length Chk1, containing N-terminal GST tag, expressed in sf9 cells.

Molecular Weight: Chk1 demonstrates a single 88 kDa band by SDS-PAGE analysis.

Purity: Chk1 is greater than 80% pure as determined by SDS-PAGE analysis.

Substrates: Chk1 phosphorylates a number of substrates, including p53, Cdc25A, Cdc25B and Cdc25C.

Inhibitors: Staurosporine and UCN-01, a derivative of staurosporine.

Unit Definitions: One unit is defined as the amount of kinase required to incorporate 1 nmol of phosphate into the GST-Cdc25C (167-267), per minute at 30°C.

Assay Conditions: Assay activity of Chk1 in a 50 μ L reaction containing 20 mM Hepes KOH (pH 7.5), 5 mM MgCl₂, 1 mM DTT, 100 μ M [γ -³²P] ATP (1 μ Ci), and 4 μ g of GST-Cdc25C fusion protein. Start the reaction by adding 10 μ L of the enzyme, diluted 50-fold in a buffer containing 20 mM Hepes KOH (pH 7.5), 1 mM DTT, 0.03 % Brij35. Incubate for 30 minutes at 30°C. Terminate the reaction by adding 600 μ L of cold 10 % TCA solution containing 0.2 % sodium pyrophosphate and stand on ice for 15 min. Filtrate acid insoluble material through GFC filters (Whatman Inc.), wash 4 times with 1 % TCA and rinse filters with ethanol. Dry filters and count in a liquid scintillation counter.

Storage and Stability: Stable for 12 months at -70°C from date of shipment. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap. Aliquot enzyme to avoid repeated freezing and thawing.

Related Products:

*Checkpoint Kinase Assay/Inhibitor Screening Kit -1: Cat# CY-1162

*Chk2 Positive Control: Cat# CY-E1162-2

*C-TAK1 Positive Control: Cat# CY-E1162-3



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General References:

1. Graves PR, *et al.*, *J Biol Chem.* **275**(8):5600-5, 2000.
2. Furnari B, *et al.*, *Mol Biol Cell.* **10**(4):833-45, 1999.
3. Liu Q, *et al.*, *Genes Dev.* **14**(12):1448-59, 2000.
4. Graves PR, *et al.*, *J Biol Chem.* **275**(8):5600-5, 2000.

PRODUCED BY

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