Chk2 Positive Control

(Human, full length, recombinant enzyme expressed in *E. coli.*)

Cat# CY-E1162-2

Lot No.
For 200 Assays
2 units (0.005 units / µL)

Product Description: Human full length Chk2, containing a *N*-terminal GST tag, expressed in *E. coil*. Purified by GSH agarose chromatography. The Chk2 Positive control is designed to use for Checkpoint Kinase Assay /Inhibitor Screening Kit-1 [Cat# CY-1162]. The Chk2 Positive Control should be added to the well at 10 m units/well. For instance, diluted positive control 1:5, use 10 µL for 1 assay. Unused Chk2 Positive control should be stored at -70°C.

Product Size: Full length Chk2: 2 units/400 µL

Formulation: The Chk2 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 Chk2, containing *N*-terminal GST tag, expressed in *E. coli.*

Molecular Weight: Chk2 demonstrates a single 96kDa band by SDS-PAGE analysis.

Purity: Chk2 is greater than 85 % pure as determined by SDS-PAGE analysis.

Substrates: Chk2 phosphorylates a number of substrates, including p53, Cdc25A, Cdc25B and Cdc25C.

Inhibitors: Specific Chk2 inhibitor has not been discovered yet.

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 Chk2 in a 50 µL reaction containing 20 mM Hepes KOH (pH 7.5), 5 mM $MgCl_2$, 1 mM DTT, 100 µM [gamma ^{32}P] ATP (1 µCi), and 4 µg of GST-G Cdc25C fusion protein. Start the reaction by adding 10µL 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 µL of cold 10 % TCA solution containing 0.2 % sodium pyrophosphate and stand on ice for 15 min. Filtrate acid insoluble material through GFC filters (Whattman 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
*Chk1 Positive Control: Cat# CY-E1162-1
*C-TAK1 Positive Control: Cat# CY-E1162-3

General References:
1. Matsuoka, S., *et al*., *Science* **282:** 1893-1897,1998.
2. Matsuoka S., *et al*., *Proc Natl Acad Sci U S A.* **97**(19):10389-94, 2000.
3. Falck J, *et al*., *Nature.* **410**:842-7, 2001.
4. Bartek J & Lukas J., *Cancer Cell.* **3**(5):421-9, 2003.
5. Ahn J, *et al*., *J Biol Chem*. **278**(23):20480-9, 2003.

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