Plk1 Positive Control

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

Cat# CY-E1163

Lot No.
For 200 Assays
(0.02 units / µL x 100 µL)

Product Description: Constitutive active form of human full length Plk1, in which there is one mutation T210D in an active loop of kinase domain, containing a *N*-terminal GST tag, expressed in *E. coil*. Purified by GSH agarose chromatography. The Plk1 Positive control is designed to use for Plk1 Assay/Inhibitor Screening Kit [Cat# CY-1163]. The Plk1 Positive Control should be added to the well at 0.1 m units/well. For instance, diluted positive control 1:2000, use 10 µL for 1 assay. Unused Plk1 Positive control should be stored at -70°C.
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Product Size: Full length Plk1: 2 units/100 µL

Formulation: The Plk1 Positive Control is supplied frozen in a buffer containing 20mM Hepes-KOH (pH 7.5), 1 % BSA, 1mM EDTA, 1 mM DTT, 50mM NaCl, 0.03 % Brij35 and 50% glycerol.

Source: Human full length Plk1, containing *N*-terminal GST tag, expressed in *E. coli*.

Molecular Weight: Plk1 demonstrates a single 92 kDa band by SDS-PAGE analysis.

Purity: Plk1 is greater than 85 % pure as determined by SDS-PAGE analysis.

Substrates: Plk1 phosphorylates a number of substrates, including Cyclin B, MBP.

Inhibitors: Specific Plk1 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 alpha-casein per minute at 30°C.

Assay Conditions: Assay activity of Plk1 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 alpha-casein. 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:
*Plk1 Assay/Inhibitor Screening Kit: Cat# CY-1163

General References:
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