



Cdc2-Cyclin B Positive Control
Product Data Sheet

For Research Use Only, Not for use in diagnostic procedures

Cdc2-Cyclin B Positive Control
(Human, recombinant protein expressed in Sf9)
Cat# CY-E1164

Lot No.
For 200 Assays
(6 mUnits / μL x 500 μL)

Product Description:

Human Cdc2-Cyclin B complex expressed in recombinant baculoviruses infected sf21 cells. Purified by sequentially using phospho-cellulose and Ni-NTA agarose chromatography. The Cdc2-Cyclin B Positive control is designed to use for CycLex Cdc2-Cyclin B Assay Kit (Cat# CY-1164). The Cdc2-Cyclin B Positive Control should be added to the well at 15 m units/well. For instance, diluted positive control 1:4, use 10 μL for 1 assay. Unused Cdc2-Cyclin B Positive control should be stored at -70°C .

Product Size: Recombinant Cdc2-Cyclin B: 3 units/500 μL

Formulation: The Cdc2-Cyclin B 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 Cdc2-Cyclin B containing N-terminal His tag, expressed in sf9 cells.

Molecular Weight: Cdc2-Cyclin B Positive Control demonstrates a double 34 and 62 kDa bands by SDS-PAGE analysis.

Purity: Cdc2-Cyclin B Positive Control is greater than 70% pure as determined by SDS-PAGE analysis.

Substrates: Cdc2-Cyclin B phosphorylates a number of substrates, including Histone H1, Rb and Cdc7.

Inhibitors: Puruvanol A and Alsterpaullone.

Unit Definition: One unit is defined as the amount of kinase required to incorporate 1nmol of phosphate into the GST-Cdc7 per minute at 30°C .

Assay Conditions: Assay activity of Cdc2-Cyclin B in a 50 μL reaction containing 20 mM Hepes KOH (pH 7.5), 5 mM MgCl_2 , 1 mM DTT, 100 μM [γ - ^{32}P] ATP (1 μCi), and 4 μg of GST-GST-Cdc7 fusion protein. Start the reaction by adding 10 μL 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 (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.



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Related Products:

* Cdc2-Cyclin B Assay Kit: Cat# CY-1164

* Anti-phospho-Cdc7-Thr376 monoclonal antibody (TK-3H7): Cat# CY-M1021

References:

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