cGK Positive Control (Catalytic domain)

(Human, a.a.324-671, recombinant protein expressed in Sf9 cells)

Cat# CY-E1161-1

Lot No.
For 200 Assays
400 units (4 units/µL)

Product Description: The catalytic domain of human cGK (cGMP-Dependent Protein Kinase), containing an N-terminal GST tag and a C-terminal His tag, is expressed in recombinant baculovirus infected Sf9 cells and purified by sequentially using GSH agarose and Ni-NTA agarose chromatography. The cGK Positive Control (Catalytic domain) is designed to use for CycLex Cyclic GMP dependent protein kinase (cGK) Assay Kit (Cat# CY-1161). The cGK Positive Control (Catalytic domain) should be added to the well at 2 units/well. This enzyme shows protein kinase activity in the absence of cGMP under the protocol described below. Unused cGK Positive Control (Catalytic domain) should be stored at -70°C.

Product Size: 400 units/100 µL

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

Source: Human cGMP-Dependent Protein Kinase, 324-671, containing an N-terminal GST tag and a C-terminal His tag, expressed in Sf9 cells.

Molecular Weight: 64 kDa band by SDS-PAGE analysis.

Purity: > 90 % pure as determined by SDS-PAGE analysis.

Substrates: cGMP-Dependent Protein Kinase phosphorylates a number of substrates, including histone proteins H2b and H1, brain G protein and high mobility group 14 protein.

Inhibitors: Polycations, such as poly-L-arginine, inhibit cGMP-Dependent Protein Kinase.

Unit Definition: One unit is defined as the amount of kinase required to incorporate 1pmol of phosphate into the GST-G substrate fusion protein, per minute at 30°C.

Assay Conditions: Assay activity of cGMP-Dependent Protein Kinase 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 substrate fusion protein in the absence of cGMP. 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 (Whatman Inc.), wash 4 times with 1 % TCA and rinse filters with ethanol. Dry filters and count in a liquid scintillation counter.

Storage Conditions: 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.

References:

1. Edelman, A.M., Blumenthal, D.K. and Krebs, E.G. Protein serine/threonine kinases. *Ann. Rev. Biochem*. 56, 567, 1987
2. Beebe, S.J. and Corbin, J.D. In: *The Enzymes*, Vol. 17, 3rd ed., Boyer, P.D. and Krebs, E.D., eds., 44, 1986
3. Corbin, J.D. and Doskeland, S.O. *J. Biol. Chem*. 258, 11391, 1983
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