



cGK Positive Control (Full length) (Bovine, full length, recombinant protein expressed in Sf9 cells) Cat# CY-E1161-2

Lot No. For 200 Assays 400 units (8 units/µL)

Product Description: The full length of Bovine cGK (cGMP-Dependent Protein Kinase) is expressed by baculovirus system and purified by conventional column chromatography. The cGK Positive Control (Full length) is designed to use for CycLex Cyclic GMP dependent protein kinase (cGK) Assay Kit (Cat# CY-1161). The cGK Positive Control (Full length) should be added to the well at 2 units/well. This enzyme shows protein kinase activity in the presence of cGMP under the protocol described below. Unused cGK Positive Control (Full length) should be stored at -70°C.

Product Size: 400 units/50 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: Bovine cGMP-Dependent Protein Kinase, full length, expressed in Sf9 cells.

Molecular Weight: 75 kDa band by SDS-PAGE analysis.

Purity: > 80 % 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 Definitions: One unit is defined as the amount of kinase required to incorporate 1 pmol of phosphate into the GST-G substrate fusion protein in the presence of $10 \mu M cGMP$, per minute at $30^{\circ}C$.

Assay Conditions: Assay activity of cGMP-Dependent Protein Kinase in a 50 μ L reaction containing 20 mM Hepes KOH (pH 7.5), 5 mM MgCl₂, 1 mM DTT, 100 μ M [gamma ³²P] ATP (1 μ Ci), 10 μ M cGMP and 4 μ g of GST-G substrate fusion protein in the absence of cGMP. 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 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.



cGK Positive Control (Full length) Product Data Sheet



For Research Use Only, Not for use in diagnostic procedures

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
- 4. Endo, S et al. Proc. Natl. Acad. Sci. USA. 96, 2467-2472, 1999

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