



cGK Positive Control (Full length)

Product Data Sheet

For Research Use Only, Not for use in diagnostic procedures

cGMP Dependent Protein Kinase Positive Control (Full length) (Bovine, recombinant protein expressed in Sf9) Cat# CY-E1161-2

Lot No.
For 200 Assays
(8 units / μL x 50 μL)

Product Description: Bovine full length cGMP-Dependent Protein Kinase expressed by baculovirus system and purified by conventional column chromatography. The cGMP Dependent Protein Kinase Positive Control (full length) is designed to use for CycLex cGK Assay Kit [Cat# CY-1161]. The cGMP Dependent Protein Kinase Positive Control (full length) should be added to the well at 2 units/well. For instance, diluted positive control 1:40, use 10 μL for 1 assay. Unused cGMP Dependent Protein Kinase Positive Control should be stored at -70°C .

The cGMP Dependent Protein Kinase Positive Control (catalytic domain) [Cat# CY-E1161-1] is also available from CycLex.

Product Size: Full length cGMP-Dependent Protein Kinase (I α -Isozyme) 400 units/50 μL

Formulation: The cGMP Dependent Protein Kinase 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: Recombinant cGMP-Dependent Protein Kinase full length is purified from recombinant baculovirus infected Sf9 cells. This enzyme shows protein kinase activity in the presence of cGMP under the protocol described below.

Molecular Weight: cGMP-Dependent Protein Kinase demonstrates a single 75kDa band by SDS-PAGE analysis.

Purity: cGMP-Dependent Protein Kinase is greater than 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 μM cGMP, per minute at 30°C .

Assay Conditions: Assay activity of cGMP-Dependent Protein Kinase (full length) 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), 10 μM cGMP and 4 μg of GST-G substrate fusion protein in the absence of cGMP. 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



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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: Store cGMP-Dependent Protein Kinase frozen at -70°C , where it is stable for at least 12 months from date of purchase. Avoid multiple freeze-thaw cycles. If thawed, store at 4°C if entire vial will be used within two weeks; or refreeze aliquots in liquid nitrogen and store at -70°C .

Related Products:

* Cyclic GMP dependent protein kinase (cGK) Assay Kit): Cat# CY-1161

* cGMP Dependent Protein Kinase Positive Control (catalytic domain): Cat# CY-E1161-1

References

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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
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