cGMP Dependent Protein Kinase Positive Control (catalytic domain)

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

Cat# CY-E1161-1

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

Product Description: Human catalytic domain of cGMP-Dependent Protein Kinase containing a *N*-terminal GST tag and a C-terminal His tag, expressed in recombinant baculovirus infected sf9 cells. Purified by sequentially using GSH agarose and Ni-NTA agarose chromatography. The GMP Dependent Protein Kinase Positive Control (catalytic domain) is designed to use for CycLex cGK Assay Kit [Cat# CY-1161]. The GMP Dependent Protein Kinase Positive Control (catalytic domain) should be added to the well at 2 units/well. For instance, diluted positive control 1:200, use 10 µL for 1 assay. Unused GMP Dependent Protein Kinase Positive should be stored at -70°C.

The GMP Dependent Protein Kinase Positive Control (full length) [Cat# CY-E1161-2] is also available from CycLex.

Product Size: Catalytic domain of cGMP-Dependent Protein Kinase (I alpha-Isozyme) 4000 units/100 µL

Formulation: The GMP 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 catalytic domain is purified from recombinant baculovirus infected Sf9 cells. This enzyme shows protein kinase activity in the absence of cGMP under the protocol described below.

Molecular Weight: cGMP-Dependent Protein Kinase demonstrates a single 64 kDa band by SDS-PAGE analysis.

Purity: cGMP-Dependent Protein Kinase is greater than 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 (catalytic domain) 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 DDT, 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 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 (full length): Cat# CY-E1161-2

References
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