Certificate of Analysis

PKG Kinase Enzyme System:

Cat.# V4248

Part# 9PIV4248

Revised 4/18

Includes:

Part No.	Name	Size
V517A	cGMP-Dependent Protein Kinase (α isozyme)	6,000 units
V745A	cGMP-Dependent Protein Kinase Peptide Substrate (10mg/ml)	1mg
V307C	5X Reaction Buffer A	1.5ml
V641A	cGMP, 1mM	500µl
P117A	DTT, 100mM	100µl

Description: cGMP-Dependent Protein Kinase (PKG) is purified from bovine lung according to the method of Corbin and Døskeland (1). PKG is a serine/threonine protein kinase present in smooth muscle and a variety of other tissues, including lung, heart and Purkinje cells of the cerebellum (2,3). The gene sequence accession number is NP_776861.1.

Storage Buffer: 10mM potassium phosphate (pH 6.8), 1mM EDTA, 25mM β-mercaptoethanol, 150mM NaCl and 12% sucrose.

Storage Conditions: Avoid multiple freeze-thaw cycles or exposure to frequent temperature changes. These fluctuations can greatly alter product stability. For optimal storage, dispense PKG into smaller aliquots upon receipt and store at < –65°C; store the rest of the components at –20°C. The enzyme is stable for 1 month at 4°C. See the expiration date on the Product Information Label.

Unit Definition: One unit is the amount of kinase required to incorporate 1pmol of phosphate into the test heptapeptide, RKRSRAE, per minute at 30°C. See Activity Assay in the Quality Control Assays section for buffer and conditions (4).

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Quality Control Assays

Activity Assay: cGMP-Dependent Protein Kinase activity is determined in an assay buffer containing 97.2µg/ml heptapeptide substrate, 40mM Tris-HCl (pH 7.5), 20mM magnesium acetate, 0.2mM ATP, 2.86µM cGMP and 30,000cpm/µl γ-[^{32}P] ATP. The reaction is run for 10 minutes at 30°C and terminated by spotting onto P81 filters.

Molecular Weight: cGMP-Dependent Protein Kinase demonstrates a 78kDa band by SDS-PAGE analysis.

Protein Concentration: Determined by Bradford Assay using BSA as a standard.

Purity: Greater than 90% as determined by SDS-PAGE analysis and Coomassie® blue staining.

Stimulation: Kinase activity is stimulated more than threefold by addition of 2.86µM cGMP.

References

1. Corbin, J.D. and Døskeland, S.O. (1983) Studies of two different intrachain cGMP-binding sites of cGMP-dependent protein kinase. *J. Biol. Chem.* **258**, 11391–7.
2. Edelman, A.M., Blumenthal, D.K. and Krebs, E.G. (1987) Protein serine/threonine kinases. *Ann. Rev. Biochem.* **56**, 567–613.
3. Beebe, S.J. and Corbin, J.D. (1986) In: *The Enzymes*, Vol. 17, 3rd., Boyer, P.D. and Krebs, E.D., eds.
4. Roskoski, R. (1983) Assays of protein kinase. *Methods Enzymol.* **99**, 3–6.

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Usage Information

Protocol

The ADP-Glo™ Kinase Assay can be used to measure the activity of PKG by quantifying the amount of ADP produced during a kinase reaction. The following is only a brief protocol. For more detailed and complete protocols, see the *ADP-Glo™ Kinase Assay Technical Manual #*TM313 found at: **www.promega.com/protocols**, and Kinase Enzyme Systems Protocol at: **www.promega.com/KESProtocol**

1. **Kinase Reaction:** In a 384-well low-volume plate (5µl total reaction volume) or 96-well plate (25µl total reaction volume), perform PKG kinase reaction using 1X Reaction buffer A supplemented with 50µM DTT and containing the following:
   - Kinase (desired amount)
   - 1µg of cGMP-Dependent Protein Kinase Peptide Substrate
   - 2.86µM cGMP
   - ATP (see concentration in Application Note available on the protocol page for Cat.# V4248*)
   - Inhibitor (or DMSO for control)

   Incubate at room temperature for 30–60 minutes or any desired time.
2. **ADP-Glo™ Kinase Assay:**
   - Add 5µl of ADP-Glo™ Reagent. (Add 25µl if using a 96-well plate.)
   - Incubate at room temperature for 40 minutes.
   - Add 10µl of Kinase Detection Reagent. (Add 50µl if using a 96-well plate.)
   - Incubate at room temperature for 30–60 minutes.
   - Record luminescence (Integration time: 0.5–1 second).

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