

Product Contents

cGMP-Dependent Protein Kinase (Alpha Isozyme):

Part No.	Size
V517A	6,000u

Description: cGMP-Dependent Protein Kinase 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 (1,2). This kinase is implicated in the regulation of smooth muscle relaxation, platelet function, sperm metabolism, cell division and nucleic acid synthesis (1). The kinase is a 78kDa polypeptide consisting of a regulatory domain and a catalytic domain and is active as a homodimer. Cellular substrates include histone proteins, the type 1 regulatory subunit of cAMP-Dependent Protein Kinase, and brain G protein, a novel protein found in the mammalian cerebellum.

Storage Buffer: cGMP-Dependent Protein Kinase is supplied frozen in storage buffer containing 10mM potassium phosphate (pH 6.8), 1mM EDTA, 25mM β -mercaptoethanol, 150mM NaCl and 12% sucrose.

Storage Conditions: For long-term storage, store at -70°C . For daily or weekly use, dispense into small aliquots. Once thawed, store at 4°C . The enzyme is stable for one month at 4°C . Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.

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 conditions, below, for buffer and conditions (3).

Part# 9PIV517

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Quality Control Assays

Activity Assay Conditions

cGMP-Dependent Protein Kinase activity is determined in an assay buffer containing 97.2 $\mu\text{g/ml}$ heptapeptide substrate, 40mM Tris-HCl (pH 7.5), 20mM magnesium acetate, 0.2mM ATP, 2.86 μM cGMP, 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. Protein concentration is listed on product label.

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

Stimulation: Kinase activity is stimulated more than 3-fold by addition of 2.86 μM cGMP.

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I. Additional Information and Standard Applications

Source: cGMP-Dependent Protein Kinase is purified from bovine lung according to the method of Corbin and Doskeland (4).

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.

Activators: cGMP-Dependent Protein Kinase is activated by high nanomolar levels of cGMP. Other cyclic compounds, such as cAMP and cIMP, activate the kinase at micromolar concentrations. β -mercaptoethanol is required for activity.

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

Use cGMP-Dependent Protein Kinase to investigate the role of target protein phosphorylation in cell regulation and signal transduction. Applications include:

- Microinjection studies of the role of cGMP-Dependent Protein Kinase in cellular processes
- In vitro enzymological studies of protein phosphorylation by the enzyme
- Determination of cGMP-Dependent Protein Kinase substrates and inhibitors
- Determination of stoichiometry and location of phosphorylation of cGMP-Dependent Protein Kinase substrates

II. References

1. Edelman, A.M., Blumenthal, D.K. and Krebs, E.G. (1987) *Ann. Rev. Biochem.* **56**, 567–613.
2. Beebe, S.J. and Corbin, J.D. (1986) In: *The Enzymes*, Vol. 17, 3rd., Boyer, P.D. and Krebs, E.D., eds.
3. Roskoski, R. (1983) Assays of protein kinase. *Meth. Enzymol.* **99**, 3–6.
4. Corbin, J.D. and Doskeland, S.O. (1983) *J. Biol. Chem.* **258**, 11391–7.

III. Related Products

Product	Size	Cat.#
Protein Kinase C	1 μ g	V5261
cAMP-Dependent Protein Kinase, Catalytic Subunit	2,500u	V5161
Casein Kinase I	100u	V5631
Casein Kinase II	100u	V5621
cGMP-Dependent Protein Kinase Peptide Substrate	1mg	V7451
DNA-Dependent Protein Kinase	2,500u	V5811
EGF Receptor	10u	V5551
cdc2 Kinase, Human, Recombinant	100u	V4781