Certificate of Analysis

cAMP-Dependent Protein Kinase, Catalytic Subunit:

Part No.	Size
V516A	2,500u

Part# 9PIV516

Revised 11/10

Description: The purified 40kDa cAMP-Dependent Protein Kinase (PKA), Catalytic Subunit, supplied by Promega may be used to phosphorylate target proteins or to study in vitro enzymological cascades of neural and hormonal signal transduction (1–3). Intracellular targets include ion channels (4), transcriptional activator proteins (5), and regulatory enzymes of glycogen metabolism (1). This enzyme does not require cAMP for activity.

Source: Recombinant *E. coli* strain expressing the catalytic subunit of bovine PKA.

Storage Buffer: 350mM potassium phosphate (pH 6.8) and 0.1mM DTT.

Storage Conditions: See the Product Information Label for storage recommendations and expiration date.

Unit Definition: One unit is the amount of enzyme required to incorporate 1pmol of phosphate into casein in one minute. The assay buffer is 40mM Tris-HCl (pH 7.4), 20mM magnesium acetate, 0.2mM ATP and 30,000cpm/µl [γ-[^{32}P] ATP]. Please see product label for lot-specific information.

Quality Control Assays

Activity Assay: cAMP-Dependent Protein Kinase activity is determined in a 60µl reaction containing 40mM Tris-HCl (pH 7.4), 20mM magnesium acetate, 0.2mM [γ-^{32}P]ATP (500–1,000cpm/pmol) and 130µM Kemptide. The reaction is incubated for 5 minutes at 30°C and is terminated by spotting 40µl of the reaction mix onto Whatman® P-81 filters and soaking in 0.5% H_3PO_4 for 5 minutes. Following a total of 5 H_3PO_4 washes of 5 minutes each, the filters are rinsed with ethanol, dried and counted. A value of 200pmol obtained for kemptide phosphorylation under these conditions corresponds to 1pmol of phosphorylated casein.

Purity: 90%, as estimated by SDS-PAGE analysis and Coomassie® staining.

Protein Concentration: Determined by Bradford Assay using BSA as a standard. See product label for lot specific information.

Signed by: J. Stevens

J. Stevens, Quality Assurance

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Usage Information

I. Description

cAMP-Dependent Protein Kinase (PKA) is an ubiquitous serine/threonine protein kinase present in a variety of tissues, including brain, skeletal muscle and heart tissues. Changes in intracellular cAMP levels regulate cellular responses by influencing interaction between the Regulatory (R) and Catalytic (C) Subunits of PKA (6). The PKA holoenzyme exists as an inactive tetrameric complex (R_2C_2), which consists of a regulatory dimer (R_2) associated with two Catalytic Subunits. When cAMP binds to R_2, the tetramer dissociates, forming R_2 • $cAMP_4$ and two active Catalytic Subunits, which can then phosphorylate a wide variety of intracellular target proteins. The free regulatory dimer has no known enzymatic activity and is characterized by cAMP binding and inhibition of the Catalytic Subunit.

PKA plays an important role in regulating glycogen metabolism. In response to hormone-induced increases in intracellular cAMP levels, PKA phosphorylates glycogen synthetase (inhibiting its activity) and phosphorylase kinase, thereby blocking glycogen synthesis and enhancing the net breakdown of glycogen (1).

The purified 40kDa cAMP-Dependent Protein Kinase (PKA), Catalytic Subunit, supplied by Promega may be used to phosphorylate target proteins or to study in vitro enzymological cascades of neural and hormonal signal transduction (1–3). Intracellular targets include ion channels (4), transcriptional activator proteins (5), and regulatory enzymes of glycogen metabolism (1). This enzyme does not require cAMP for activity.

II. Assay Conditions

Assay activity of the Catalytic Subunit for 5 minutes at 30°C in a 60µl reaction containing 40mM Tris-HCl (pH 7.4), 20mM magnesium acetate, 0.2mM [γ-^{32}P]ATP (500–1,000cpm/pmol) and 130µM Kemptide. Terminate the reaction by spotting 40µl of the reaction mix onto Whatman® P-81 filters and soaking in 0.5% H_3PO_4 for 5 minutes. Perform a total of 5 H_3PO_4 washes (5 minutes each) and rinse filters with ethanol. Dry filters and count.

III. Related Products

Product	Size	Cat.#
PepTag® Non-Radioactive cAMP-Dependent Protein Kinase Assay	120 reactions	V5340
SignaTECT® cAMP-Dependent Protein Kinase Assay System	96 reactions	V7480
ProFluor® PKA Assay	4 plate	V1240
	8 plate	V1241
Kinase-Glo® Luminescent Kinase Assay	10ml	V6711
	10 × 10ml	V6712
	100ml	V6713
	10 × 100ml	V6714
Kinase-Glo® Plus Luminescent Kinase Assay	10ml	V3771
	10 × 10ml	V3772
	100ml	V3773
	10 × 100ml	V3774
cAMP-Dependent Protein Kinase Peptide Inhibitor	1mg	V5681
Kemptide Peptide Substrate	1mg	V5601
InCELLect™ AKAP St-Ht31 Inhibitor Peptide	150µl	V8211
InCELLect™ St-Ht31P Control Peptide	150µl	V8221
cAMP	500µl	V6421

IV. References

1. Cohen, P. (1978) The role of cyclic-AMP-dependent protein kinase in the regulation of glycogen metabolism in mammalian skeletal muscle. *Curr. Top. Cell. Regul.* **14**, 117–96.
2. Ruegg, J.C. and Paul, R.J. (1982) Vascular smooth muscle. Calmodulin and cyclic AMP-dependent protein kinase after calcium sensitivity in porcine carotid skinned fibers. *Circ. Res.* **50**, 394–9.
3. Osterrieder, W. *et al.* (1982) Injection of subunits of cyclic AMP-dependent protein kinase into cardiac myocytes modulates Ca^{2+} current. *Nature* **298**, 576–8.
4. Rossie, S. and Catterall, W.A. (1987) Cyclic-AMP-dependent phosphorylation of voltage-sensitive sodium channels in primary cultures of rat brain neurons. *J. Biol. Chem.* **262**, 12735–44.
5. Montminy, M.R. and Bilezikjian, L.M. (1987) Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. *Nature* **328**, 175–8.
6. Flockhart, D.A. and Corbin, J.D. (1982) Regulatory mechanisms in the control of protein kinases *C.R.C. Crit. Rev. Biochem.* **12**, 133–86.

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