

Certificate of Analysis

cAMP-Dependent Protein Kinase, Catalytic Subunit:

Part No.	Size
V516A	2,500u

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/ μ l [γ - 32 P] ATP]. Please see product label for lot-specific information.

Part# 9PIV516

Revised 8/16



AF9PIV516 0816V516

Quality Control Assays

Activity Assay: cAMP-Dependent Protein Kinase activity is determined in a 60 μ l reaction containing 40mM Tris-HCl (pH 7.4), 20mM magnesium acetate, 0.2mM [γ - 32 P]ATP (500–1,000cpm/pmol) and 130 μ M Kemptide. The reaction is incubated for 5 minutes at 30°C and is terminated by spotting 40 μ l of the reaction mix onto Whatman® P-81 filters and soaking in 0.5% H₃PO₄ for 5 minutes. Following a total of 5 H₃PO₄ washes of 5 minutes each, the filters are rinsed with ethanol, dried and counted. A value of 200cpm 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.



Promega

Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	www.promega.com

PRODUCT USE LIMITATIONS, WARRANTY, DISCLAIMER

Promega manufactures products for a number of intended uses. Please refer to the product label for the intended use statements for specific products. Promega products contain chemicals which may be harmful if misused. Due care should be exercised with all Promega products to prevent direct human contact.

Each Promega product is shipped with documentation stating specifications and other technical information. Promega products are warranted to meet or exceed the stated specifications. Promega's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Promega makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, PRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO PROMEGA PRODUCTS. In no event shall Promega be liable for claims for any other damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Promega products to perform in accordance with the stated specifications.

Kinase-Glo, PepTag, ProFluor and SignaTECT are registered trademarks of Promega Corporation. InCELLect is a trademark of Promega Corporation.

Coomassie is a registered trademark of Imperial Chemical Industries, Ltd. Whatman is a registered trademark of Whatman Paper Company, Ltd.

© 2010, 2016 Promega Corporation. All Rights Reserved.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

Part# 9PIV516
Printed in USA. Revised 8/16.

Signed by:

R. Wheeler, Quality Assurance

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 $R_2 \cdot cAMP_4$ 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
	10ml	V6711
Kinase-Glo® Luminescent Kinase Assay	10 × 10ml	V6712
	100ml	V6713
	10 × 100ml	V6714
	10ml	V3771
Kinase-Glo® Plus Luminescent Kinase Assay	10 × 10ml	V3772
	100ml	V3773
	10 × 100ml	V3774
	1mg	V5681
cAMP-Dependent Protein Kinase Peptide Inhibitor	1mg	V5601
Kemptide Peptide Substrate	150µl	V8211
InCELLect™ AKAP St-Ht31 Inhibitor Peptide	150µl	V8211
InCELLect™ St-Ht31P Control Peptide	500µl	V6421
cAMP		

IV. References

- 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.
- 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.
- 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.
- 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.
- 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.
- 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.