

Product Contents

PPase-2A:

| Part No. | Size (units) |
|----------|--------------|
| V631A | 25 |

Description: Protein Phosphatase-2A (PPase-2A) is a serine/threonine phosphatase isolated from human red blood cells. It is isolated as a heterodimer of 60kDa (A) and 36kDa (C) subunits. PPase-2A has the ability to dephosphorylate the α -subunit of phosphorylase kinase (1). With its 36–38kDa catalytic subunit, PPase-2A has broad substrate specificity and may play a regulatory role in DNA replication, transcription, protein synthesis, mitosis and glycogen metabolism (2). PPase-2A is stimulated in vitro by basic proteins such as protamine, histones and polylysine (1). The enzyme is inhibited by several environmental toxins and tumor promoters such as okadaic acid (3) and microcystin-LR (4). The chemically synthesized phosphopeptide, RRA(pT)VA (available in the Promega Ser/Thr Phosphatase Assay System, Cat.# V2460), is an excellent substrate for PPase-2A.

Storage Buffer: 20mM MOPS (pH 7.5), 150mM NaCl, 1mM MgCl₂, 1mM EGTA, 0.1mM MnCl₂, 1mM DTT, 0.1mg/ml BSA, 60mM 2-mercaptoethanol, 50% glycerol.

Storage Conditions: See the Product Information Label for storage recommendations. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. The performance of this product is guaranteed for six months from the date of purchase if stored and handled properly.

Unit Definition: One unit is the amount of PPase-2A required to release 1nmol of phosphate from PNPP in one minute at 30°C under the assay conditions described below.

Part# 9PIV631

Revised 3/06



AF9PI V631 0306V631



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 |

Quality Control Assays

Activity Assay Conditions: A 100 μ l reaction volume contains 50mM Tris-HCl (pH 8.5), 20mM MgCl₂, 1mM DTT, and 14mM PNPP. Reactions are incubated for 15 minutes at 30°C.

Concentration: 0.5u/ μ l.

Identity: The identity of PPase-2A Catalytic Subunit is confirmed by performing an activity assay in the presence and absence of PPase-1 Inhibitor-2 (50nM), a specific inhibitor for type 1 protein phosphatases. In the presence of 50nM PPase-1 Inhibitor-2 the enzyme activity is inhibited by less than 10%.

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.

© 2002, 2006 Promega Corporation. All Rights Reserved.

Costar is a registered trademark of Corning, Inc.

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# 9PIV631
Printed in USA. Revised 3/06

I. PNPP Activity Assay

The following assay can be used to verify the activity of Promega's PPase-2A Catalytic Subunit. One unit of PPase-2A will release 1nmol of inorganic phosphate per minute at 30°C. PPase-2A activity also may be assayed using ³²P-labeled phosphorylase *a* as a substrate (5) or by using the Promega non-radioactive Ser/Thr Phosphatase Assay System (Cat.# V2460; 6).

The amount of PPase-2A activity added per reaction should not exceed 0.02nmol of PNPP converted per minute in this assay. Above this rate, the reaction becomes nonlinear. Thus, serial dilutions are necessary to obtain an accurate value of activity.

PPase-2A is inhibited by low concentrations of okadaic acid (IC₅₀ = 1nM, with complete inhibition at 5–10nM). This property can be used to identify PPase-2A activity, since 10nM okadaic acid has less of an effect on other known protein phosphatases [IC₅₀ = 10–15nM for PPase-1, IC₅₀ = 5μM for PPase-2B. PPase-2C is insensitive to okadaic acid (7,8)]. PPase-2A is not inhibited by nanomolar concentrations of PPase-1 Inhibitor-1 or Inhibitor-2 (2,7).

A. Protocol

Materials to Be Supplied by the User

(Solution compositions are provided in Section II.)

- reaction buffer
- enzyme dilution buffer
- stop solution
- *p*-nitrophenyl phosphate (PNPP) (MW = 263.1; Sigma Cat.# N2765)
- enzyme dilution buffer containing 10nM okadaic acid [optional (see Step 2);

1. Thaw an aliquot (7.6ml) of reaction buffer and add 40mg of PNPP (final concentration = 14.2mM). Mix by gentle vortexing and store on ice.
2. Immediately before performing the assay, prepare a serial dilution (1 to 20, 1 to 40 and 1 to 60) of the purified enzyme in enzyme dilution buffer.

Optional: To obtain evidence that the phosphatase activity is attributable to PPase-2A, prepare a second set of enzyme dilutions in enzyme dilution buffer containing 10nM okadaic acid.

3. Determine the total number of reactions to be performed. Perform duplicate reactions if desired. (Create one "no enzyme" control for each enzyme dilution.) For each reaction, add 90μl of reaction buffer containing PNPP to one well of a 96-well plate. Preincubate the plate for 5 minutes at 30°C in a shallow water bath.
4. To start the reactions, add 10μl of each sample. Samples are: a) diluted enzyme, b) no enzyme (control reactions), or c) diluted enzyme containing 10nM okadaic acid (**optional**, see note following Step 2). Incubate for 15 minutes at 30°C.
5. Stop the reaction by adding 25μl of stop solution to each well. Mix well.
6. Dry the outside of the 96-well plate and read the absorbance at 410nm.

B. Calculation of Enzyme Activity

Enzyme activity (nmol P_i/min/μl) =

$$\frac{(\text{assay volume})(\text{dilution factor})(\text{sample absorbance}_{410\text{nm}} - \text{control absorbance}_{410\text{nm}})}{(\text{sample volume})(\text{reaction time})(\text{extinction coefficient})(\text{path length})}$$

Where:

Assay volume = 125μl

Dilution factor = fold dilution of enzyme

Sample volume = 10μl

Reaction time = 15 minutes

Extinction coefficient of *p*-nitrophenolate (pH 7.4) = 17.5/cm[nmol/μl]

Path length = 0.4cm*

*This is the path length for standard plates (i.e., Costar® 3590 or Fisher 08-758-15). The path length should be measured if different plates are used.

II. Composition of Buffers and Solutions

enzyme dilution buffer

| | |
|----------|----------------------|
| 20mM | MOPS (pH 7.5) |
| 150mM | NaCl |
| 60mM | β-mercaptoethanol |
| 1mM | MgCl ₂ |
| 1mM | EGTA |
| 0.1mM | MnCl ₂ |
| 1mM | DTT |
| 0.1mg/ml | bovine serum albumin |
| 10% | glycerol |

reaction buffer

| | |
|------|-------------------|
| 50mM | Tris-HCl (pH 8.5) |
| 20mM | MgCl ₂ |
| 1mM | DTT |

Store 7.6ml aliquots at –20°C.

stop solution

1M NaOH

III. Related Products

| Product | Size | Cat.# |
|--|--------------|-------|
| Serine/Threonine Phosphatase Assay System | 96 reactions | V2460 |
| Tyrosine Phosphatase Assay System | 96 reactions | V2471 |
| cAMP-Dependent Protein Kinase, Catalytic Subunit | 2,500u | V5161 |
| Kemptide (PKA) Peptide Substrate | 1mg | V5601 |
| PPase-2B | 10u | V6361 |

IV. References

1. Tung, H.Y., Alemany, S. and Cohen, P. (1985) The protein phosphatases involved in cellular regulation. 2. Purification, subunit structure and properties of protein phosphatases-2A0, 2A1, and 2A2 from rabbit skeletal muscle. *Eur. J. Biochem.* **148**, 253–263.
2. Shenolikar, S. (1994) Protein serine/threonine phosphatases—new avenues for cell regulation. *Annu. Rev. Cell Biol.* **10**, 55–86.
3. Bialojan, C. and Takai, A. (1988) Inhibitory effect of a marine-sponge toxin, okadaic acid, on protein phosphatases. Specificity and kinetics. *Biochem. J.* **256**, 283–290.
4. Yoshizawa, S. *et al.* (1990) Inhibition of protein phosphatases by microcystins and nodularin associated with hepatotoxicity. *J. Cancer Res. Clin. Oncol.* **116**, 609–614.
5. DeGuzman, A. and Lee, E.Y.C. (1988) Preparation of low-molecular-weight forms of rabbit muscle protein phosphatase. *Meth. Enzymol.* **159**, 356–368.
6. *Serine/Threonine Phosphatase Assay System Technical Bulletin #TB218*, Promega Corporation.
7. Cohen, P. (1989) The structure and regulation of protein phosphatases. *Annu. Rev. Biochem.* **58**, 453–508.
8. Peruski, L.F., Jr., Wadzinski, B.E. and Johnson, G.L. (1993) Analysis of the multiplicity, structure, and function of protein serine/threonine phosphatases. *Adv. Prot. Phosphatases* **7**, 9.