Product Manual

Phosphate Assay Kit (Fluorometric)

Catalog Number

STA-686 **1000 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Phosphorus exists as a soluble free phosphate ion in biological systems and is often referred to as inorganic phosphate (P_i). Phosphate is found as organic phosphate in a number of biological molecules such as ATP, DNA, protein, and phospholipids. Many metabolic processes are regulated by phosphate such as amino acid metabolism, activation of proteins, carbon metabolism, enzymatic cell signaling and energy transfer.

Cell Biolabs' Phosphate Assay Kit is a simple fluorometric assay that measures the amount of total inorganic phosphate present in solutions, foods or biological samples in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 1000 assays, including blanks, phosphate standards, endogenous controls*, and unknown samples. Sample phosphate concentrations are determined by comparison with a known phosphate standard. The kit has a detection sensitivity limit of 0.78 µM phosphate.

Assay Principle

Cell Biolabs' Phosphate Assay Kit (Fluorometric) measures total inorganic phosphate in solutions, cell culture supernatants, lysates, or food samples. In the presence of P_i, maltose is converted to glucose and glucose-1-phosphate by maltose phosphorylase. Glucose is then converted to D-gluconic acid and hydrogen peroxide by glucose oxidase. The resulting hydrogen peroxide is detected with a highly specific fluorescence probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples are compared to a known concentration of phosphate standard within the 96-well microtiter plate format. Samples and standards are incubated then read with a standard 96-well fluorometric plate reader (Figure 1).

**Note: Endogenous levels of glucose, maltose and maltase can interfere with the assay. Therefore an endogenous control must be run for each sample to account for potential interference of these molecules.*

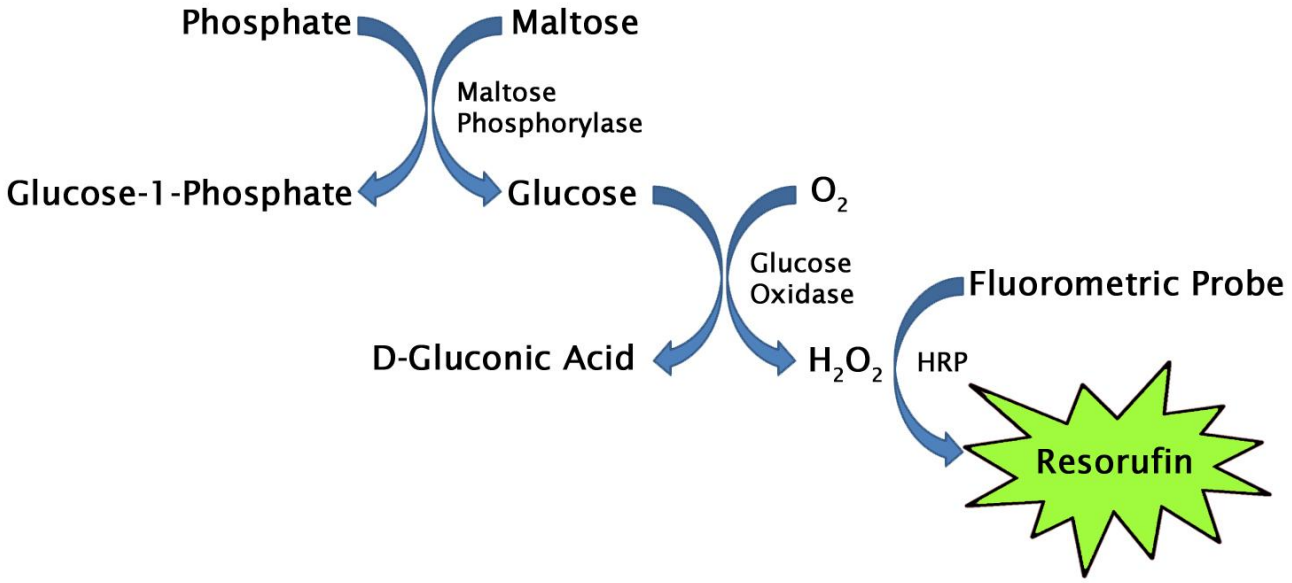

Figure 1. Phosphate assay principle.

Related Products

1. STA-398: Free Glycerol Assay Kit (Colorimetric)
2. STA-399: Free Glycerol Assay Kit (Fluorometric)
3. STA-670: Homocysteine ELISA Kit
4. STA-671: S-Adenosylhomocysteine (SAH) ELISA Kit
5. STA-672: S-Adenosylmethionine (SAM) ELISA Kit
6. STA-671-C: S-Adenosylmethionine (SAM) ELISA and S-Adenosylhomocysteine (SAH) ELISA Combo Kit
7. STA-674: Glutamate Assay Kit
8. STA-675: Hydroxyproline Assay Kit
9. STA-680: Glucose Assay Kit (Colorimetric)
10. STA-681: Glucose Assay Kit (Fluorometric)

Kit Components

Box 1 (shipped at room temperature)

1. Phosphate Standard (Part No. 268501): One 200 µL tube of 50 mM sodium phosphate dibasic pH 7.4 in water
2. 10X Assay Buffer (Part No. 267401): Two 25 mL bottles
3. Fluorometric Probe (Part No. 268601): One 500 µL tube
4. HRP (Part No. 268506): One 100 µL tube of a 100 U/mL solution in glycerol
5. Maltose (Part. No. 268505): One 1 mL tube of 40 mM Maltose in 100 mM Tris pH 7.4

Box 2 (shipped on blue ice packs)

1. Maltose Phosphorylase (Part No. 268503): One 1 mL tube at 200 U/mL

   Note: One unit is defined as the amount of enzyme that will produce 1 micromole of D-Glucose from Maltose per minute at pH 7.0 at 30°C.

2. Glucose Oxidase (Part No. 268504): One 500 µL tube at 200 U/mL

   Note: One unit is defined as the amount of enzyme that will oxidize 1.0 micromole of beta-D-glucose to D-gluconic acid and hydrogen peroxide per minute at pH 5.1 at 35°C.

Materials Not Supplied

1. Distilled or deionized water
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips

4. Standard 96-well fluorescence black microtiter plate and/or black cell culture microplate
5. Multichannel micropipette reservoir
6. Fluorescence microplate reader capable of reading excitation in the 530-570 nm range and emission in the 590-600 nm range.

Storage

Upon receipt, store the 10X Assay Buffer at room temperature. Store all other components at -20ºC. The Fluorescence Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

- 1X Assay Buffer: Dilute the 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity.
- Reaction Mix: Prepare Reaction Mix by diluting the Fluorometric Probe 1:100, HRP 1:500, Glucose Oxidase 1:100, Maltose Phosphorylase 1:50, and Maltose 1:100 in 1X Assay Buffer. For example, add 50 µL Fluorometric Probe stock solution, 10 µL HRP stock solution, 50 µL of Glucose Oxidase, 100 µL of Maltose Phosphorylase, and 50 µL of Maltose to 4.74 mL 1X Assay Buffer for a total of 5 mL. The above example is enough for ~100 assays. The Reaction Mix is stable for 1 day at 4ºC.

  Note: Scale down the described example as needed and prepare only enough for immediate use
- Endogenous Control Mix: Prepare Endogenous Control Mix (no Maltose Phosphorylase) by diluting the Fluorometric Probe 1:100, HRP 1:500, Glucose Oxidase 1:100, and Maltose 1:100 in 1X Assay Buffer. For example, add 50 µL Fluorometric Probe stock solution, 10 µL HRP stock solution, 50 µL of Glucose Oxidase, and 50 µL of Maltose to 4.84 mL 1X Assay Buffer for a total of 5 mL. The above example is enough for ~100 assays. The Endogenous Control Mix is stable for 1 day at 4ºC.

  Note: Scale down the described example as needed and prepare only enough for immediate use.

Preparation of Samples

- Cell culture supernatants: Cell culture media formulated with phosphate should be avoided. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The cell conditioned media can be assayed directly or diluted as necessary. Prepare the Phosphate standard curve in the same non-conditioned media lacking phosphate as described above.
  Note: Maintain pH between 7 and 8 for optimal working conditions as the Fluorometric Probe is unstable at high pH (>8.5).
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in buffer containing 50 mM Tris pH 7.4 and 150 mM NaCl. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates can be assayed undiluted or diluted as necessary in 1X Assay Buffer.
- Tissue lysates: Sonicate or homogenize tissue sample in buffer containing 50 mM Tris pH 7.4 and 150 mM NaCl and centrifuge at 10000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.

Notes:

- *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
- *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Fluorometric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 5).*
- *Avoid samples containing DTT or β-mercaptoethanol since Resorufin is not stable in the presense of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh Phosphate standards before use by diluting in 1X Assay Buffer. Dilute the 50 mM Phosphate Standard 1:2 with distilled water to 25 mM final concentration. Use the 25 mM Phosphate Standard to prepare a series of the remaining Phosphate standards according to Table 1 below.

Standard Tubes	25 mM Phosphate Solution (µL)	1X Assay Buffer (µL)	Phosphate (µM)
1	4	1996	50
2	250 of Tube #1	250	25
3	250 of Tube #2	250	12.5
4	250 of Tube #3	250	6.25
5	250 of Tube #4	250	3.13
6	250 of Tube #5	250	1.56
7	250 of Tube #6	250	0.78
8	0	250	0

Table 1. Preparation of Phosphate Standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each sample replicate requires two paired wells, one positive well and one endogenous control well.
2. Add 50 µL of each standard into wells of a fluorescence black microtiter plate.
3. Add 50 µL of each unknown sample to each of two separate wells.
4. Add 50 µL of Reaction Mix to all standard wells and one half of the paired sample wells.
5. Add 50 µL of Endogenous Control Mix to the remaining paired sample wells.
6. Mix all well contents thoroughly and incubate for 2 hours at 37ºC protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.

7. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.
8. For each unknown sample, subtract the RFU from the Endogenous Control Mix from the RFU from the Reaction Mix.
9. Calculate the concentration of phosphate within samples by comparing the sample signal to the standard curve.

Example of Results

The following figures demonstrate typical Phosphate Assay Kit (Fluorometric) results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

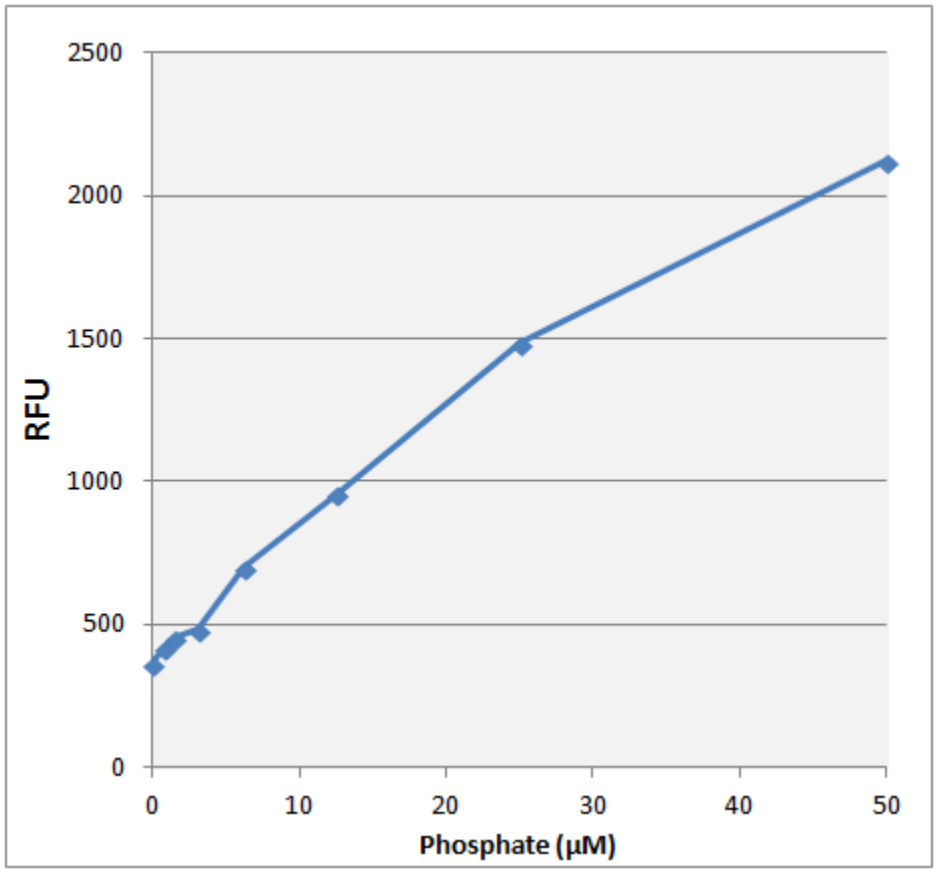

Figure 2: Phosphate Standard Curve.

References

1. Dick C.F, Dos-Santos A.L.A, and Meyer-Fernandes J.R. (2011) *Enz. Res.* **103980:**1-7.
2. Ping W., Ma L., Hou X., Wang M., Wu Y., Liu F., and Deng X.W. (2003) *Plant Physiol.* **132**: 1260-1271.
3. Marschner H (1995) Mineral *Nutr. HigherPlants* , **2nd Ed**:889pp.
4. Safian M.F. and Lehmann W.D. (2015) *Anal. Bioanal. Chem.* **407**: 2933-2937.

5. Votyakova TV, and Reynolds IJ (2001) *Neurochem*. **79**:266.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com