PhosphoWorks™ Colorimetric Phosphate Assay Kit

Blue Color

Ordering Information:
Product Number: #21665 (1000 assays)

Instrument Platform:
Absorbance microplate reader or spectrophotometer

Storage Conditions:
Keep at 4 °C and avoid light

Introduction

Cells utilize a wide variety of phosphate and polyphosphate esters as enzyme substrates, second messengers, membrane structural components and vital energy reservoirs. Phosphate is involved in many biological processes. For example, phosphatases, ATPases and several other enzymes catalyze biochemical reactions in which inorganic phosphate (Pi) is released from a phosphoester substrate. Detection of many phosphoester–metabolizing enzymes is difficult because suitable substrates are not available. It usually has been necessary to determine inorganic phosphate release using tedious colorimetric assays or radioisotope-based methods. This PhosphoWorks™ Colorimetric Phosphate Assay Kit has been developed for measuring the activity of any Pi-generating enzyme using a modified Malachite Green formulation. The kit provides sensitive detection of Pi, an alternative to hazardous radioactive methods and other less sensitive colorimetric assays. The measurement of Pi is based on the change in absorbance of MG Plus™ in the presence of molybdate. Unlike other Malachite Green formulations, this kit gives a completely stable end-point signal that is not prone to precipitation. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required.

Kit Features

Broad Applications: Can be used for monitoring any biological processes that either generate or consume phosphate.

Convenient: Formulated to have minimal hands-on time.

Non-Radioactive: No special requirements for waste treatment.

Use of Native substrates: Substrates can be proteins, peptides, nucleotides, sugars, organic molecules or inorganic salts.

Kit Components

Component	Amount	Storage Conditions
Component A: 1 mM KH_2PO_4	1 vial (1 mL)	4 °C
Component B: MG Plus™ Reagent	1 bottle (20 mL)	4 °C and avoid light

Assay Protocol (for 1 96-well plate)

Brief Summary

Prepare test samples (80 µL) along with phosphate standard dilutions (80 µL) from component A → Add component B (20 µL)→ Incubate at room temperature for 10-40 min→ Read Absorbance at 600-660 nm

1. Prepare assay reagents

Cautions: *Avoid phosphate-containing buffers when preparing your samples!*

1.1 Warm all components to room temperature before use.

1.2 We strongly recommend that clear microplates or cuvettes be used for achieving the best results.

2. Prepare Phosphate standards and test samples:

2.1 Add 50 µL of 1 mM phosphate standard (component A) in 950 µL of deionized water or enzyme reaction buffer to get 50 µM phosphate solution.

2.2 Take 200 µL of 50 µM phosphate solution to perform 1:2 serial dilutions to get 25, 12.5, 6.25, 3.125, 1.56, and 0.78 µM standard phosphate solutions.

2.3 Add phosphate-containing test samples into a 96-well clear microplate as described in Tables 1 and 2

Table 1. Layout of phosphate standards and test samples in a clear 96-well microplate:

BL	BL	TS	TS	….	….						
PS1	PS1	….	….	….	….						
PS2	PS2										
PS3	PS3										
PS4	PS4										
PS5	PS5										
PS6	PS6										
PS7	PS7										

Note: PS=Phosphate standards, BL=Blank control, TS=test samples.

Table 2. Reagent composition for each well:

Phosphate Standard	Blank Control	Test Sample
Serial dilutions* (80 µL)	Phosphate-free water or buffer (80 µL)	80 µL

*Note: *Add the serially diluted phosphate standards from 0.1 µM to 50 µM into wells from PS1 to PS7 in duplicate.*

3. Run PhosphoWorks™ Colorimetric Phosphate Assay

3.1 Shake Component B (MG Plus™ Reagent) well before use. Add 20 µL/well of Component B to the wells of phosphate standards, blank control, and test samples. Mix the reagents completely.
Note: For a 384-well plate, add 40 µL sample and 10 µL Component B (MG Plus™ Reagent) per well.

3.2 A blue-green color will develop in the phosphate-containing wells in 10 to 40 min. Measure absorbance at 600-660 nm on a microplate reader or a spectrophotometer.
Note 1: At high phosphate concentration (>100 µM), precipitates may form. Dilute your samples and redo the assays.
Note 2: For cuvette assay that requires the total volume larger than 100 µL, you can either multiple the volume of sample and Component B (MG Plus™ Reagent) proportionally or dilute the final reaction mixture with 1 M H_2OS_4 or 1 M HCl before measuring the absorption.

4. Data Analysis

The absorption (OD reading) in blank wells (with water or buffer only) is used as a control, and is subtracted from the values for those wells with the phosphate standards and test samples. A typical set of data is shown in Figure 1 (phosphate standard curve). Calculate the phosphate concentration of the samples according to the phosphate standard curve.

Note: The phosphate standard curve is used to calibrate for the variation of different instruments and for different assay conditions.

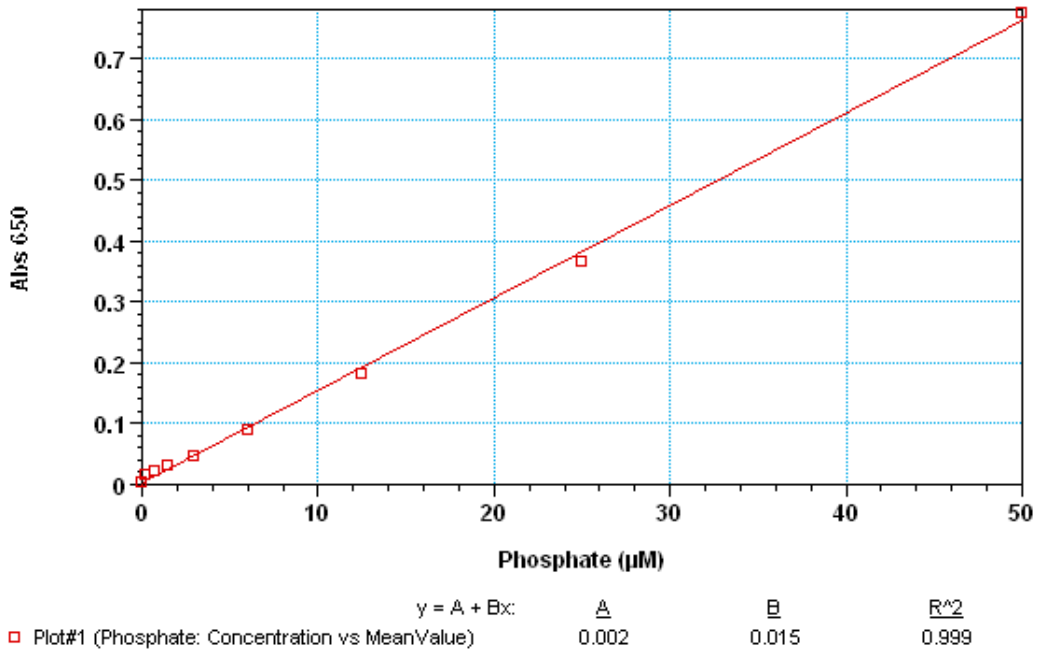

Figure1. Phosphate dose response on 96-well clear plate using a SpectraMax Plus microplate reader (Molecular Devices) measured with the PhosphoWorks™ Clorimetric Phosphate Assay Kit*Blue Color*. As low as 0.1µM phosphate can be detected with 10 min incubation time.

Potential Applications

This kit might be used for the following applications:

- A variety of ATPases
- Actin polymerization
- Aminoacyl-tRNA synthetase
- Aspartate transcarbamylase
- Dethiobiotin synthetase
- Glycerol kinase
- Glycogen phosphorylase
- GTPases
- *myo*-Inositol monophosphatase
- Phospholysine and phosphohistidine phosphatases
- Phosphorylase *a* phosphatase
- Phosphorylase kinase
- Serine phosphatase
- Self-assembly of actin and tubulin
- Tyrosine protein phosphatases

Related Products

21664	PhosphoWorks™ Colorimetric Phosphate Assay Kit *Yellow Color*	1 kit
21655	PhosphoWorks™ Fluorimetric ADP Assay Kit *Red Fluorescence*	1 kit
21658	PhosphoWorks™ Fluorimetric Phosphate Assay Kit *Blue Fluorescence*	1 kit
21660	PhosphoWorks™ Fluorimetric Phosphate Assay Kit *Red Fluorescence*	1 kit
21611	PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit	1 kt
21612	PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit *Green Fluorescence*	1 kt
21610	PhosphoWorks™ Luminometric ATP Assay Kit *Bright Glow*	1 kit
21609	PhosphoWorks™ Luminometric ATP Assay Kit *Steady Glow*	1 kit
21659	PhosphoWorks™ MESG Phosphate Assay Kit *Colorimetric*	1 kit

References

1. Bernal C, Palacin C, Boronat A, Imperial S. (2005) A colorimetric assay for the determination of 4-diphosphocytidyl-2-C-methyl-D-erythritol 4-phosphate synthase activity. Anal Biochem, 337, 55.
2. Hannig C, Hamkens A, Becker K, Attin R, Attin T. (2005) Erosive effects of different acids on bovine enamel: release of calcium and phosphate in vitro. Arch Oral Biol, 50, 541.
3. Mahuren JD, Coburn SP, Slominski A, Wortsman J. (2001) Microassay of phosphate provides a general method for measuring the activity of phosphatases using physiological, nonchromogenic substrates such as lysophosphatidic acid. Anal Biochem, 298, 241.
4. Cala SE. (1999) Determination of a putative phosphate-containing peptide in calreticulin. Biochem Biophys Res Commun, 259, 233.