PhosphoWorks[™] Colorimetric Phosphate Assay Kit *Blue Color*

Ordering Information	Storage Conditions	Instrument Platforms
Product Number: 21665 (1000 Assays)	Keep at 4 °C and protect from light	Spectrophotometer

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. The detection of many phosphoester– metabolizing enzymes is difficult because suitable substrates are not available. The tedious radioisotope-based method is usually used to determine inorganic phosphate release.

This PhosphoWorks[™] Colorimetric Phosphate Assay Kit has been developed to measure the activity of any Pi-generating enzyme using a modified Malachite Green formulation. It provides sensitive detection of Pi, an alternative to hazardous radioactive methods. The measurement of Pi is based on absorbance change of MG Plus[™] in the presence of molybdate. Unlike other Malachite Green formulations, this kit gives a completely stable endpoint signal that is not prone to precipitation. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. This assay is non-continuous while kit 21660 is a continuous assay. It is also complementary to kit 21659 that uses MESG as a continuous Pi indicator.

Kit Key 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

Components	Amount
Component A: 1 mM KH ₂ PO ₄	1 vial (1 mL)
Component B: MG Plus [™] Reagent	1 bottle (20 mL)

Assay Protocol (for 1 96-well plate)

Brief Summary

Prepare test samples (80 µL) along with phosphate standard dilutions (80 µL) from 1 mM KH₂PO₄ (Component A) \rightarrow Add MG PlusTM Reagent (Component B) (20 µL) \rightarrow Incubate at room temperature for 10 - 40 minutes

1. Prepare assay reagents:

- Caution: Phosphate-containing buffers should be avoided when preparing the samples!
- 1.1 Warm all components at room temperature before use.
- 1.2 It is strongly recommend that clear microplates or cuvettes be used to achieve the best results.

2. Prepare serially diluted phosphate standards and/or 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 a 50 μM phosphate standard solution.
- 2.2 Take 200 μL of 50 μM phosphate standard solution to perform 1:2 serial dilutions to get 25, 12.5, 6.25, 3.125, 1.56, and 0.78 μM serially diluted phosphate standards.
- 2.3 Add phosphate-containing test samples into a clear 96-well microplate as described in Tables 1 and 2.

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

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

Note: PS=*Phosphate Standards, BL*=*Blank Control, TS*=*Test Samples.*

Table 2.	Reagent	composition	for	each	well
	0				

Phosphate Standard	Blank Control	Test Sample
Serial Dilutions*: 80 µL	Phosphate-free water or buffer: $80 \ \mu L$	80 μL

Note: *Add the serial dilutions of phosphate standard from 0.1 μ M to 50 μ M into wells from PS1 to PS7 in duplicate.

3. Run PhosphoWorks[™] colorimetric phosphate assay:

3.1 Shake MG Plus[™] Reagent (Component B) well before use. Add 20 µL/well of MG Plus[™] Reagent (Component B) into the wells of phosphate standards, blank control, and test samples. Mix the reagents thoroughly.

Note: For a 384-well plate, add 40 μ L of sample and 10 μ L of MG PlusTM Reagent (Component B) into each well.

3.2 A blue-green color will develop in the phosphate-containing wells in 10 to 40 minutes. Monitor absorbance at 600-660 nm with an absorbance microplate reader or a spectrophotometer.

Note 1: At high phosphate concentration (> 100 \muM), precipitates may form. Dilute your samples and redo the assays.

Note 2: For cuvette assay that requires the total volume larger than 100 μ L, either multiple the volume of sample and MG PlusTM Reagent (Component B) proportionally or dilute the final reaction mixture with 1 M H₂OS₄ or 1 M HCl before measuring the absorption.

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 of those wells with the phosphate standards and test samples. A phosphate standard curve is shown in Figure 1. Calculate the phosphate concentrations of the samples according to the phosphate standard curve. *Note: The phosphate standard curve is used to calibrate the variation of different instruments and different assay conditions.*



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

References

- 1. Bernal C, Palacin C, Boronat A, Imperial S. (2005) A colorimetric assay for the determination of 4diphosphocytidyl-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.

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