PhosphoWorks™ Colorimetric Phosphate Assay Kit *Blue Color*

Catalog number: 21665
Unit size: 1000 Tests

Component	Storage	Amount
Component A: 1 mM KH2PO4 Standard	Refrigerate (2-8 °C), Minimize light exposure	1 vial (1 mL)
Component B: MG Plus™ Reagent	Refrigerate (2-8 °C), Minimize light exposure	1 bottle (20 mL)

OVERVIEW

Phosphate is involved in many biological reactions. For example, phosphatases, ATPases and several other enzymes catalyze reactions in which inorganic phosphate (Pi) is released from a substrate. This PhosphoWorks™ Phosphate Assay Kit has been developed for measuring the activity of any Pi-generating enzyme. The kit is formulated to give sensitive detection of Pi, providing an alternative to hazardous radioactive methods and other less sensitive colorimetric assays. The measurement of Pi is based on the change in absorbance of a malachite green derivative in the presence of molybdate. Unlike other malachite dye formulations, this kit gives a completely stable end-point signal that is not prone to precipitation.

AT A GLANCE

Protocol summary

1. Prepare test samples or Phosphate standards (80 µL)
2. Add MG Plus™ Reagent (Component B) (20 µL)
3. Incubate at room temperature for 10 - 40 minutes
4. Monitor absorbance at 600 - 660 nm or spectrophotometer

Important Phosphate-containing buffers should be avoided when preparing the samples. To achieve the best results, it is strongly recommend to use clear microplates or cuvettes. Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument: Spectrophotometer
Absorbance: 600 - 660 nm
Recommended plate: Clear bottom

Instrument: Absorbance microplate reader
Absorbance: 600 - 660 nm
Recommended plate: Clear bottom

PREPARATION OF STANDARD SOLUTION

Phosphate standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/21665

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 standard solution (PS7). Take 50 µM Phosphate standard solution (PS7) and perform 1:2 serial dilutions to get serially diluted Phosphate standards (PS6 - PS1) with deionized water or enzyme reaction buffer.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Phosphate standards and test samples in a clear 96-well microplate. PS=Phosphate Standards (PS1 - PS7, 0.78 to 50 µM), BL=Blank Control, TS=Test Samples.

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

Table 2. Reagent composition for each well.

Well	Volume	Reagents
PS1 - PS7	80 µL	Serial Dilutions (0.78 to 50 µM)
BL	80 µL	Phosphate-free water or buffer
TS	80 µL	test sample

1. Prepare Phosphate standards (PS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 40 µL of reagent per well instead of 80 µL.
2. Shake MG Plus™ Reagent (Component B) well before use.
3. Add 20 µL of MG Plus™ Reagent (Component B) to each well of Phosphate standard, blank control, and test samples to make the total Phosphate assay volume of 100 µL/well. Mix the reagents thoroughly. For a 384-well plate, add 10 µL of MG Plus™ Reagent (Component B) into each well instead, for a total volume of 50 µL/well.
4. A blue-green color will develop in the phosphate-containing wells in 10 to 40 minutes. Monitor absorbance with an absorbance microplate reader at 600 - 660 nm or a spectrophotometer.

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

Note For cuvette assay that requires the total volume larger than 100 µL, either multiple the volume of sample and MG Plus™ Reagent (Component B) proportionally or dilute the final reaction mixture with 1 M H_2SO_4 or 1 M HCl before measuring the absorption.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a

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standard curve and equation. This equation can be used to calculate Phosphate samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

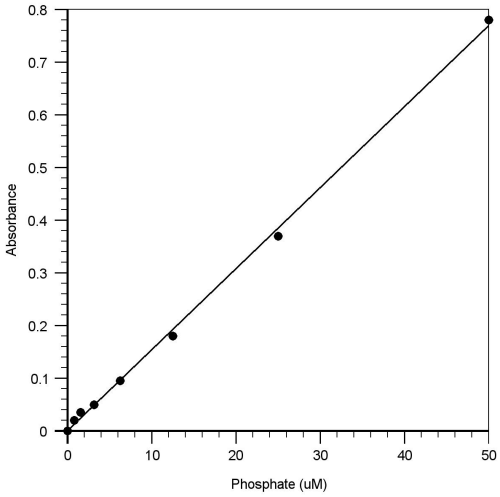

Figure 1. Phosphate dose response was measured with PhosphoWorks™ Colorimetric Phosphate Assay Kit on a clear 96-well plate using a SpectraMax Plus microplate reader (Molecular Devices).

DISCLAIMER