Phosphate Assay Kit (Colorimetric)

Catalog Number KA6676

1000 assays

Version: 02

Intended for research use only

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Introduction

Background

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.

Principle of the Assay

The Phosphate Assay Kit (Colorimetric) 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.

General Information

Materials Supplied

List of component

Component	Amount
Component A: KH_2PO_4 Standard (1 mM)	1 mL
Component B: MG Plus Reagent	20 mL

Storage Instruction

Store components in refrigerate (2-8 °C), minimize light exposure.

Precautions for Use

For research use only.

Assay Protocol

Reagent Preparation

✓ Phosphate standard

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.

Assay Procedure

✓ Key Parameters

Instrument	Spectrophotometer
Absorbance	600 – 660 nm
Recommended plate	Clear bottom
Instrument	Absorbance microplate reader
Absorbance	600 – 660 nm
Recommended plate	Clear bottom

✓ 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

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

Assay protocol

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.

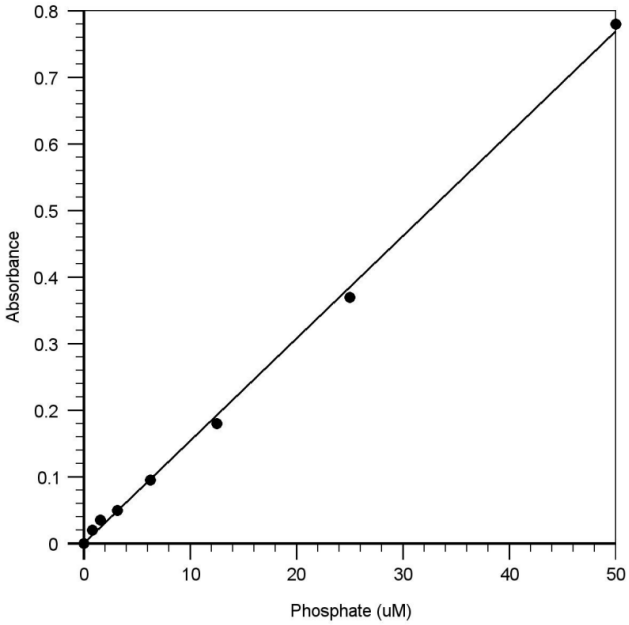
4. A blue-green color will develop in the phosphate-containing wells in 10 to 40 minutes. Monitor absorbance with an absorbance microplate reader at 600 - 660 nm or a spectrophotometer.
   Note: At high phosphate concentration (>100 µM), precipitates may form. Dilute your samples and redo the assays.
   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.

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

Data Analysis

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 standard curve and equation. This equation can be used to calculate Phosphate samples.



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

References

Plate layout

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