PhosphoWorks™ Colorimetric MESG Phosphate Assay Kit *UV absorption*

Catalog number: 21659
Unit size: 200 Tests

Component	Storage	Amount
Component A: Assay Buffer	Freeze (<-15 °C)	1 bottle (10 mL)
Component B: MESG Substrate	Freeze (<-15 °C), Minimize light exposure	1 vial (lyophilized powder)
Component C: Purine Nucleoside Phosphorylase (PNP)	Freeze (<-15 °C), Minimize light exposure	1 vial (lyophilized powder)
Component D: 1 mM KH2PO4 Standard	Refrigerate (2-8 °C), Minimize light exposure	1 vial (1 mL)

OVERVIEW

In the presence of inorganic phosphate MESG is converted to 2-amino-6-mercapto-7-methlpurine by purine nucleoside phosphorylase (EC 2.4.2.1) with absorption wavelength shift to red. This feature has been used to develop our convenient MESG phosphate assay kit. Our kit provides all the essential reagents including MESG, phosphorylase and reaction buffer. The MESG substrate gives an absorbance increase at 360 nm on phosphorylysis at pH 6.5-8.5, and at pH 7.6 the change in extinction coefficient is 11,000 M-1cm-1. The assay is shown to quantitate phosphate in solution at concentrations at least down to 2 µM. It can be used to measure the kinetics of phosphate release from phosphatases (such as GTPases and ATPases) by coupling the two enzymatic reactions.

AT A GLANCE

Protocol summary

1. Prepare 50 µL of test samples and/or phosphate standards
2. Add 50 µL of working solution
3. Incubate at room temperature for 30 minutes
4. Monitor absorbance at 360 nm

Important Thaw all the four components at room temperature before use.

KEY PARAMETERS

Instrument:	Spectrophotometer
Absorbance:	360 nm
Recommended plate:	Clear UV-transparent
Instrument:	Absorbance microplate reader
Absorbance:	360 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/21659

Add 50 µL of 1 mM KH_2PO_4 (Component D) into 950 µL of deionized water or enzyme reaction buffer to get 50 µM Phosphate standard solution (PS7). Take 50 µM Phosphate standard solution and perform 1:2 serial dilutions to get serially diluted Phosphate standards (PS6 - PS1) with deionized water or enzyme reaction buffer.

PREPARATION OF WORKING SOLUTION

1. Add 500 µL of ddH_2O to the vial of MESG Substrate (Component B). Mix well by vortexing to get MESG Substrate solution.

   Note 250 µl is enough for one plate.

2. Add 100 µL of ddH_2O to the vial of Purine Nucleoside Phosphorylase (PNP; Component C). Mix well by vortexing to get Purine Nucleoside Phosphorylase solution.

3. Add the whole volume of MESG Substrate solution and Purine Nucleoside Phosphorylase solution into the bottle of Assay Buffer (Component A) and mix well to get the working solution. Place the working solution on ice.

   Note This working solution is stable for at least 4 hours on ice. It is not recommended to freeze the working solution for another assay. To achieve the desirable results, UV-transparent plates or cuvettes are required. Due to the high sensitivity of this assay to Pi, it is extremely important to use Pi-free laboratory ware.

SAMPLE EXPERIMENTAL PROTOCOL

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

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	Reagent
PS1 - PS7	50 µL	Serial Dilutions (0.78 to 50 µM)
BL	50 µL	Phosphate-free water or buffer
TS	50 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 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of working solution to each well of Phosphate standard, blank control, and test samples to make the total assay volume of 100 µL/well. Mix the reagents thoroughly. For a 384-well plate, add 25 µL of working solution into each well instead, for a total volume of 50 µL/well.

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3. Incubate at room temperature for 30 minutes.

4. Monitor the absorbance with a microplate reader or spectrophotometer at 360 nm.

Note For cuvette assay that requires the total volume larger than 100 µL, multiply the volume of sample and assay reagent proportionally 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 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

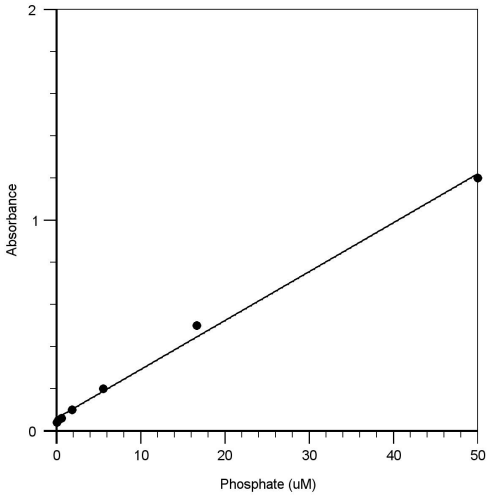

Figure 1. Phosphate dose response was measured with PhosphoWorks™ Colorimetric MESG Phosphate Assay Kit on a 96-well UV plate using a SpectraMax Plus microplate reader (Molecular Devices).

DISCLAIMER