PhosphoWorks™ Colorimetric MESG Phosphate Assay Kit**UV Absorption**

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 21659 (200 assays)	Keep in freezer and protect from light	Spectrophotometer Absorbance microplate reader

Introduction

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. It is usually necessary to determine inorganic phosphate release using tedious colorimetric assays or radioisotope-based methods.

This PhosphoWorks™ Colorimetric MESG Phosphate Assay Kit has been developed for measuring the activity of any Pi-generating enzyme using MESG reagent. The measurement of Pi is based on absorbance change of MESG by phosphate. In the presence of inorganic phosphate, MESG is converted to 2-amino-6-mercapto-7-methlpurine by purine nucleoside phosphorylase with absorption wavelength shift to red. This feature has been used to develop our convenient MESG phosphate assay kit, an alternative to hazardous radioactive methods. The MESG substrate gives an absorbance increase at 360 nm on phosphorylysis at pH 6.5-8.5. The assay is shown to quantitate phosphate at the final concentration as low as 0.2 μM. The kit has been used for monitoring ATPase activities. It can also be used for monitoring phosphatase activities.

Kit Components

Components	**Amount**
Component A: Assay Buffer	1 Bottle (10 mL)
Component B: MESG Substrate	1 vial (lyophilized powder)
Component C: Purine Nucleoside Phosphorylase (PNP)	1 vial (lyophilized powder)
Component D: 1 mM KH_2PO_4	1 vial (1 mL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare 50 μL of test samples and/or phosphate standards → Add 50 μL of assay solution → Incubate at room temperature for 30 minutes → Monitor absorbance at 360 nm

1. Prepare assay reagents:

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

1.2 Prepare MESG Substrate (Component B) Solution: 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 ul is enough for one plate *make single used aliquos and store it at -20°C Immediately.*,

1.3 Prepare Purine Nucleoside Phosphorylase (Component C) Solution: 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.

1.4 Prepare Assay Solution: Add the whole volume of MESG Substrate Solution (from Step 1.2) and Purine Nucleoside Phosphorylase Solution (from Step 1.3) into the bottle of Assay Buffer (Component A), mix well to get the assay solution. Place the assay solution on ice.
Note 1: This Assay Solution is stable for at least 4 hours on ice. It is not recommended to freeze the assay solution for another assay.
Note 2: To achieve the desirable results, UV-transparent plates or cuvettes are required.
Note 3: Due to the high sensitivity of this assay to Pi, it is extremely important to use Pi-free laboratory ware and

2. Prepare serially diluted phosphate standards and/or test samples:

2.1 Prepare Phosphate Standard: Add 50 μL of 1 mM KH_2PO_4 (Component D) into 950 μL of deionized water or enzyme reaction buffer to give 50 μM phosphate standard solution.

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

2.3 Add phosphate-containing test samples and/or phosphate standards into a clear UV-transparent 96-well microplate according to Tables 1 and 2.

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

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

Note: PS=Phosphate Standard, BL=Blank Control, TS=Test Sample.

Table 2 Reagent composition for each well

Phosphate Standard	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	Phosphate-free water or buffer: 50 µL	50 µL

**Note: Add the serial dilutions of phosphate standard from 0.1 µM to 50 µM into wells from PS1 to PS7.*

3. Run PhosphoWorks™ MESG phosphate assay:

3.1 Add 50 µL/well of Assay Solution (from Step 1.4) into the wells of phosphate standards, blank control, and test samples. Mix the reagents thoroughly.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of Assay Solution into each well.

3.2 Incubate at room temperature for 30 minutes. 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, multiple the volume of sample and assay reagent proportionally 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 for those wells with the phosphate standards and test samples. A phosphate standard curve is shown in Figure 1. Calculate the phosphate concentration 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.*

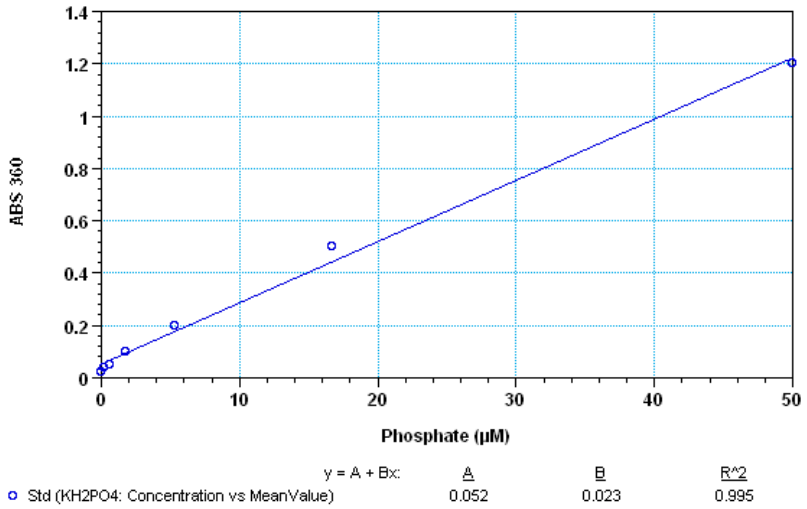

Figure1. Phosphate dose response was measured with the PhosphoWorks™ Colorimetric MESG Phosphate Assay Kit on a 96-well UV plate using a SpectraMax Plus microplate reader (Molecular Devices). As low as 0.2 µM phosphate can be detected with 30 minutes incubation.

References

1. Webb MR, Hunter JL. (1992) Interaction of GTPase-activating protein with p21ras, measured using a continuous assay for inorganic phosphate release. Biochem J, 287 (Pt 2), 555.
2. Webb MR. (1992) A continuous spectrophotometric assay for inorganic phosphate and for measuring phosphate release kinetics in biological systems. Proc Natl Acad Sci U S A, 89, 4884.