

## 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 Absorbance microplate reader

### 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 end-point 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

<b>Broad Applications:</b>	Can be used for monitoring any biological processes that either generate or consume phosphate.
<b>Convenient:</b>	Formulated to have minimal hands-on time.
<b>Non-Radioactive:</b>	No special requirements for waste treatment.
<b>Use of Native Substrates:</b>	Substrates can be proteins, peptides, nucleotides, sugars, organic molecules or inorganic salts.

### Kit Components

Components	Amount
Component A: 1 mM KH <sub>2</sub> PO <sub>4</sub>	1 vial (1 mL)
Component B: MG Plus™ Reagent	1 bottle (20 mL)

### Assay Protocol (for 1 96-well plate)

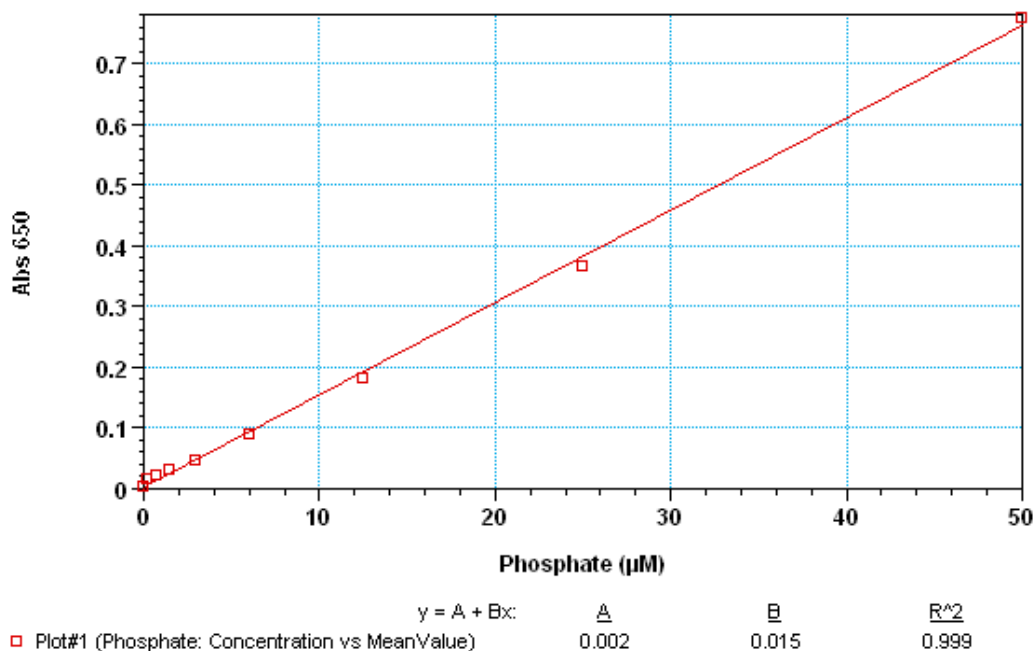
#### Brief Summary

**Prepare test samples (80 µL) along with phosphate standard dilutions (80 µL) from 1 mM KH<sub>2</sub>PO<sub>4</sub>**  
**(Component A) → Add MG Plus™ Reagent (Component B) (20 µL)**  
**→ Incubate at room temperature for 10 - 40 minutes**



## 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.*



**Figure 1.** 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

- Bernal C, Palacin C, Boronat A, Imperial S. (2005) A colorimetric assay for the determination of 4-diphosphocytidyl-2-C-methyl-D-erythritol 4-phosphate synthase activity. *Anal Biochem*, 337, 55.
- 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.
- 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.
- Cala SE. (1999) Determination of a putative phosphate-containing peptide in calreticulin. *Biochem Biophys Res Commun*, 259, 233.

**Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at [info@aatbio.com](mailto:info@aatbio.com) if you have any questions.**