SensoLyte® MG Phosphate Assay Kit
Colorimetric

Revision# 1.2	*Last Updated: July 2021*
Catalog #	**AS-71103**
Kit Size	1000 Assays (96-well plate)

- ***Convenient Format:*** Complete kit includes all the assay components.
- ***Optimized Performance:*** Optimal conditions for phosphate detection.
- ***Enhanced Value:*** Less expensive than the sum of individual components.
- ***High Speed:*** Minimal hands-on time.
- ***Assured Reliability:*** Detailed protocol is provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	1 mM Phosphate, Standard	1 mL
Component B	MG Reagent	20 mL

Other Materials Required (but not provided)

- 96-well microplate or cuvettes: Clear microplate or cuvettes.
- Microplate reader or spectrophotometer: Capable of detecting absorbance at 600-660 nm.

Storage and Handling

- Store all kit components at room temperature.

Introduction

The SensoLyte® MG Phosphate Assay Kit is based on the quantification of the blue-green complex formed between malachite green (MG), molybdate and free phosphate. The rapid color formation from the reaction can be conveniently measured on a spectrophotometer (600nm-660 nm) or on an absorbance plate reader (Figure 1). The assay involves a single reagent addition step for phosphate determination and takes only 10 minutes for color development. There is no precipitate formation, so extract filtration step is not necessary. The assay can be conveniently performed in a 96-well format for screening of phosphatase activators or inhibitors with a Z' factor of 0.7 to 0.9. It can also be performed in tubes and cuvettes by proportionally scaling up the amount of assay reagents. The assay has been widely used to quantify the liberated phosphate in phosphatase assays, lipase assays, and nucleotide triphosphatase assays.

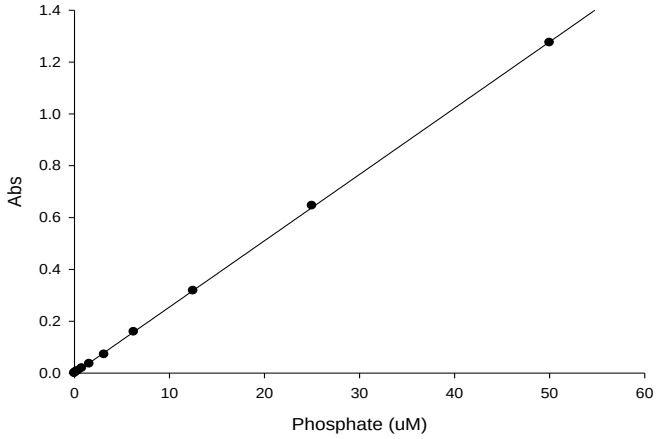

Figure 1. The linear range of MG phosphate assay

Protocol

Note: Warm all reagents to room temperature. Avoid phosphate-containing buffer when preparing your samples.

1. Prepare phosphate standard.

1.1 Phosphate standard: Add 25 µL of 1 mM phosphate standard (Component A) in 475 µL of deionized water or enzyme reaction buffer to get 50 µM phosphate solution. Then take 200 µL of 50 µM phosphate solution to perform 2-fold serial dilutions to get 25, 12.5, 6.25, 3.125, 1.56, and 0.78 µM phosphate solutions. Include a blank control.

2. Set up the phosphate assay.

2.1 Add 80 μL of test sample into microplate wells.

Note 1: Use deionized water to dilute test samples.
Note 2: If the samples are diluted in buffers containing substances that may affect assay performance, test the same amount of that buffer with phosphate standards.

2.2 Set up phosphate standard: Add 80 μL serially diluted phosphate standard solution (from Step 1.1) to the wells.

3. Run the phosphate assay.

3.1 Shake the MG reagent (Component B) well before use. Add 20 μL/well of MG reagent into each well. Mix the reagents completely by shaking the plate on a plate shaker at 100-200 rpm for 5-10 minutes.

3.2 The blue-green color will develop in the phosphate-containing wells in 10 to 40 min. Measure absorbance at 600-660 nm on a microplate reader or a spectrophotometer.

Note 1: At high phosphate concentration (>100 μM), precipitates may form. Dilute your samples and redo the assays.

Note 2: Add an equal volume of 1 N NaOH before you dispose the MG containing waste since MG contains 1 M sulfuric acid.

Note 3: When using a cuvette, which requires the total volume to be larger than 100 μL, either adjust the volume of sample and MG reagent proportionally or dilute the final reaction mixture with 1 M H_2SO_4 or 1 M HCL before measuring the absorption.

3.3 Data analysis: Refer to the Data Analysis section.

4. Data Analysis.

4.1 The absorption reading from the blank control is the background absorbance. Subtract this background reading from the readings of the other wells.

4.2 Plot the phosphate standard as Abs (absorbance unit) versus concentration (Figure 1).

Note: This phosphate standard curve is used to calibrate for the variation of different instruments and for different batches of experiments. It is also an indicator of the amount of phosphate in test samples.

4.3 Calculate the phosphate concentration of the samples according to the phosphate standard curve.