Malachite Green Phosphate Assay Kit

Introduction

The Malachite Green Phosphate Assay Kit is a kit for the detection of phosphate concentration. The principle is that under acidic conditions, malachite green, molybdate and free inorganic orthophosphate can form a green phosphomolybdate complex, and the absorbance of this green phosphomolybdate complex at 650 nm is proportional to the concentration of free inorganic phosphate in solution.

This kit can quickly, conveniently and sensitively determine free inorganic phosphate in solution, so it is often used for the determination of phosphate concentration in solution and the ATPase activity. However, this kit is only suitable for the determination of free phosphate in solution. For the bound phosphate, such as binding to lipids or proteins, certain methods must be used for release before detection. The kit has a good linear relationship in the range of 0-10 nmol during detection.

Components and Storage

Components	K2245-500 T	K2245-1000 T	K2245-2500 T
Malachite Green Reagent A	5 mL	10 mL	25 mL
Malachite Green Reagent B	10 mL	20 mL	50 mL
Phosphate Standard (10 mM)	200 μL	400 μL	1 mL

Store the kit at 4°C, stable for 1 year. Malachite Green Reagent B should be stored away from light.

Protocol

1. **Sample preparation:**

   1) Plasma, serum, and other liquid samples can be used directly for detection after preparation.

   2) For cells, collect the cells and lyse them using standard lysis procedures, then centrifuge at 12000 g, 4°C for 10 min, and use the supernatant for detection.

   3) For tissues, homogenize them using standard procedures to make a 10% tissue homogenate, then centrifuge at 12000g, 4°C for 10 min and use the supernatant for detection.

***Note:** Cell lysis or tissue homogenization should be performed on ice.

2. **Dilution of phosphate standard:** Dilute the Phosphate Standard (10 mM) with deionized water or distilled water to make a 100 μM phosphate working solution. The diluted phosphate working solution can be stably

stored at 4°C for at least one day. If other reagents are used to dilute the standard, ensure that the reagent does not have phosphate contamination. Then prepare a serial of phosphate standards according to the table below.

100μM Phosphate (μL)	ddH_2O (μL)	Phosphate (μM)	Amount Phosphate (nmol/200μL)
0	200	0	0
5	195	2.5	0.5
20	180	10	2
40	160	20	4
60	140	30	6
80	120	40	8
100	100	50	10

***Note:** Phosphates formed by divalent cations (calcium/magnesium/copper/zinc) have low solubility in water. To avoid precipitation, the diluent for the phosphate standards should not contain these cations if you use another diluent replacing deionized water or distilled water.

3. **Preparation of Colorimetric Working Solution:** Mix Malachite Green Reagent A and Malachite Green Reagent B in a 1:2 ratio to make the colorimetric working solution. If there is precipitation in the colorimetric working solution, centrifuge at 12000 rpm for 1 min and use the supernatant. The colorimetric working solution is best prepared fresh, but it can be stored in the dark at 4°C for one day.

***Note:** Malachite Green Reagent A and Malachite Green Reagent B need to be warmed to room temperature before use.

4. **Phosphate Detection：**

1) Prepare the detection system according to the table below and gently mix by pipetting, incubate at room temperature in the dark for 30 min. For better detection results, it is recommended to set up parallel or triplicate wells.

	Standard	**Sample**
serial of phosphate standards	200 μL	-
Sample	-	200 μL
Colorimetric Working Solution	30 μL	30 μL

***Note:** If the sample volume is less than 200 μL, it can be made up to 200 μL with the phosphate standards diluent, and the dilution factor of the sample should be recorded for subsequent calculation of the phosphate concentration in the sample.

2) Measure the absorbance at 650 nm with a microplate reader. After obtaining the standard curve, the phosphate concentration in the sample can be calculated.

Note

1. If this kit is accidentally stored at -20°C, causing the reagents to freeze, Malachite Green Reagent A needs to be heated to 95°C until it is completely dissolved and mixed before use. The other two solutions can be used

after regular thawing and mixing.

2. The detection wavelength of this kit is between 620-660 nm, with 650 nm being the best. If there is no microplate reader, a spectrophotometer can also be used for detection. If a spectrophotometer is used, the volume of the detection system needs to be adjusted proportionally so that the final volume meets the minimum detection volume of the colorimetric dish.
3. The sample volume in this manual is based on a 96-well plate. If a 384-well plate or colorimetric dish is used, please adjust the detection system proportionally.
4. Common laboratory cleaning agents contain high concentrations of phosphates. Before using this kit, ensure that the experimental vessels are thoroughly cleaned to prevent phosphate contamination.
5. If the detected absorbance is low, it may be due to a low phosphate content in the sample. Consider increasing the volume of the sample used within the allowable range of this kit. If the detected absorbance is high, it may be due to a high phosphate content in the sample. Consider diluting the sample appropriately before testing.
6. Malachite Green Reagent B is strongly acidic and corrosive. Please take precautions during operation to prevent direct contact with the body, and avoid corroding other vessels.
7. For your safety and health, please wear lab coats and gloves during the experiment.
8. For research use only. Not to be used in clinical diagnostic or clinical trials.

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