

Phosphate Assay Kit (Phosphomolybdic Acid Method)

P1506504

Storage 2-8°C. Protect from light.

Shipping Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

Introduction

Inorganic phosphorus reacts with molybdic acid to form phosphomolybdic acid, which is reduced to molybdenum blue by a reducing agent. The maximum absorption peak appears at 660 nm, and the absorbance is proportional to the concentration within a certain range.

Composition

P1506504	Components	48T	96T	Storage
P1506504A	Phosphomolybdic Acid Reagent A	7.5 mL	15 mL	2-8°C
P1506504B	Phosphomolybdic Acid Reagent B	0.5 mL	1 mL	2-8°C
P1506504C	Phosphomolybdic Acid Reagent C	65.4 mg	130.8 mg	2-8°C, Store in the dark
P1506504D	10 mM Standard	5 mL	10 mL	2-8°C

Usage Protocol

1. Preparation of Phosphate Standard

Prepare a 1.5 mM phosphate working solution from the 10 mM phosphate standard stock solution. Deionized water or double-distilled water is preferred as the standard diluent. The diluted working solution is stable for at least one day at 4 °C. If using self-prepared reagents for standard dilution, ensure they are free of phosphate contamination.

2. Preparation of Color Reagent

Calculate the required volume of color reagent based on the number of samples. The working concentration of Phosphomolybdic Acid Reagent C is 21.8 g/L. It can be aliquoted before dissolution according to the usage amount each time to avoid repeated freeze-thaw cycles. It is recommended to prepare freshly before use as much as possible. Phosphomolybdic acid Reagent A, Reagent B, and Reagent C are mixed at a ratio of 17:1:6. The color reagent should be stored at 4 °C, protected from light, and is stable for one day.

3. Setup of Standard Curve

NO	1.5mM Phosphate(μ L)	H ₂ O(μ L)	Phosphate(mM)
1	0	100	0
2	1.33	98.67	0.02
3	3.33	96.67	0.05
4	6.67	93.33	0.1
5	13.33	86.67	0.2
6	33.33	66.67	0.5
7	53.33	46.67	0.8
8	66.67	33.33	1
9	100	0	1.5

4. Determination of Phosphate Concentration
 - a. Add an appropriate volume of sample to a 1.5 mL centrifuge tube.
 - b. If the sample volume is less than 50 μ L, make up to 50 μ L with standard diluent and record the dilution factor.
 - c. Pipette 50 μ L of each phosphate standard (at different concentrations) and each test sample into separate wells of a 96-well plate.
 - d. Add 200 μ L of color reagent to each well.
 - e. Mix using a plate mixer or by gentle pipetting, then incubate at 37 °C for 30 minutes.
 - f. Measure the absorbance at 660 nm using a microplate reader.
 - g. Calculate the phosphate concentration of the sample based on the standard curve and the sample dilution factor.

Precautions

1. The chromogenic reagent must be prepared and used immediately. It can be stored at 4 °C for one day after preparation. The reagent must be equilibrated to room temperature before use.
2. The detection wavelength of this experiment ranges from 620 to 660 nm, with 660 nm being optimal. If a microplate reader is not available, a spectrophotometer can be used instead. In this case, adjust the volumes of the chromogenic reagent, samples and standard solutions proportionally to ensure the final volume to be tested meets the minimum detection volume of the cuvette.
3. The sample volumes described in this instruction manual are based on a 96-well plate. If cuvettes or 384-well plates are used, the volumes should be adjusted proportionally.
4. Laboratory detergents contain high concentrations of phosphate. Ensure that the experimental utensils are thoroughly cleaned to prevent phosphate contamination.
5. For better detection results, it is recommended to set up duplicate or triplicate wells for all phosphate standard solutions and samples to be tested.
6. If the measured absorbance is low, it may be due to the low phosphate content in the

sample. Consider increasing the sample dosage within the allowable range of this kit.

7. Phosphomolybdic Acid Reagent A is strongly acidic and corrosive. Handle it with care during operation, ensure effective protection to avoid direct contact with the human body, and take care to prevent corrosion of other items.
8. This product is intended for scientific research use by professionals only. It is not for clinical diagnosis or treatment, nor for use in food or pharmaceuticals, and should not be stored in ordinary residential premises.
9. For your safety and health, wear a lab coat and disposable gloves during operation.

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