

Directions For Use Phosphate Assay Kit (N164-Kit)



Kit Contents:

Phosphate Reagent A, 10ml
 Phosphate Reagent B, 50ml
 Phosphate Reagent C, 1.5ml
 Phosphate Standard, 1.0ml

INTRODUCTION

Phosphate Assay Kit is based on molybdate and malachite green dye. The rapid color formation from the reaction can be conveniently measured on a spectrophotometer (600 - 660 nm) or on a plate reader. This colorimetric assay kit has been optimized to offer superior sensitivity and prolonged shelf life. The assay is simple and fast, involving a single addition step for phosphate determination. Assays can be performed in cuvettes or conveniently in 96-well plates for high-throughput screening.

PRINCIPLE

The main principle involves the reaction of inorganic phosphate with molybdate to form a colorless unreduced phosphomolybdate complex which is converted to a blue colored complex when reduced under acidic conditions. Furthermore, phosphomolybdate gives 20 or 30 times more color when complexed with malachite green. The final product, reduced green soluble complex is measured by its absorbance at 620 nm and is a direct measure of inorganic phosphate in solution

PROCEDURE:

I. Preparation of Phosphate Standard Solution:

1. Dilute the above 0.2 M Sodium Phosphate standard 1:10,000 using distilled water. (Helpful hint: Prepare dilution by two serial dilutions of 1:100 each).
2. Prepare increment volumes (μ l) of diluted phosphate standard 100, 90, 80, 70 etc. followed by appropriate volumes of water to 100 μ l final volume in each well of a micro titer plate (see table below).

Tube/Plate Well	Neg. control	1	2	3	4	5	6	7	8	9	10
Diluted Phosphate Standard (μ l)	0	10	20	30	40	50	60	70	80	90	100
H ₂ O (μ l)	100	90	80	70	60	50	40	30	20	10	0



II. Preparation of Phosphate Assay Solution

This solution should be made immediately before use.

1. Allow Phosphate Reagent A to come to room temperature.
2. Combine Reagents A and B at a 1:4 ratio in a separate tube.
3. For every 1mL of solution from step 4, add 20uL of phosphate Reagent C to it.
4. Mix well and store at room temperature for up to 24 hours.

III. Assay Procedure:

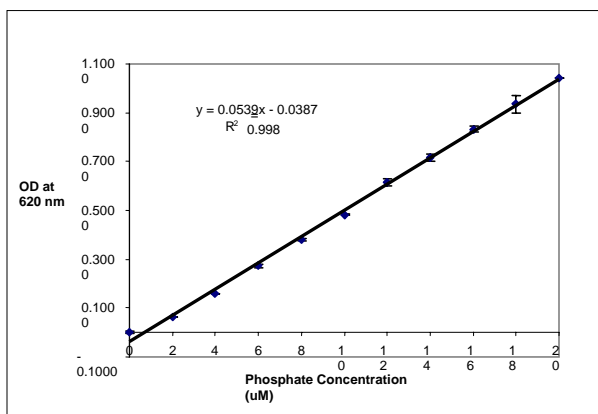
1. Add 50 µl of the combined Phosphate Assay Reagent to both the standards as well as test samples (Technical hint: Samples may need to be diluted depending on whether the total phosphate content extends beyond the standard curve generated. Some optimization may be necessary in order to obtain interpretable results).
2. Mix the samples by pipetting up and down (being careful to not introduce air bubbles to samples).
3. Samples can be read the after a 15 - 30 minute incubation at room temperature using a Micro Titer Plate Reader set to read at 620nm.

CALCULATIONS:

Subtract blanks from absorbance values. Take averages when replicates are used. Plot corrected absorbance values for the standards on the Y-axis as a function of phosphate concentration (uM) on the X-axis. Determine the slope and y-intercept using an Excel spreadsheet or other spreadsheet program capable of calculating the line equation and R² value.

EXAMPLE

Phosphate (µM) = ((A₆₂₀ - y_intercept) / Slope) X Sample dilution factor



For Research Use Only. Not for Therapeutic or Diagnostic Use.

References:

1. Lowry O H & Lopez J A. The determination of inorganic phosphate in the of labile phosphate esters. J. Biol. Chem. 162:421-8, 1946.
2. Kalckar H M. Enzymatic synthesis of a nucleoside. J. Biol. Chem. 158:723-4, 1945.
3. Fiske C H & Subbarow Y. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375-400, 1925
4. Bell R D & Doisy E A. Rapid colorimetric methods for the determination of phosphorus in urine and blood. J. Biol. Chem. 44:55-67, 1920.
5. Itaya K & Ui M. A new micromethod for the colorimetric determination of organic phosphate. Clin. Chim. Acta 14:361-6, 1966.