

Water & Soil Total Phosphate Assay Kit (Spectrophotometry)

Product Description

Total phosphate includes various forms of phosphates such as orthophosphate, metaphosphate, pyrophosphate, and polyphosphate. It reflects the phosphate levels in water and soil, serving as a critical indicator for evaluating water and soil quality.

Assay Principle

Under acidic and high-temperature conditions in the presence of a decomposing agent, both inorganic and organic phosphates are hydrolyzed into orthophosphate. Orthophosphate reacts with ammonium molybdate to form phosphomolybdic acid, which is then reduced to phosphomolybdenum blue in the presence of a reducing agent. Phosphomolybdenum blue exhibits a characteristic absorption peak at 710 nm.

Kit Components

Taking 50T/48S packing for example:

Catalog No.	Specification	Storage
CB0272S-A	10mL x 1 vial	4°C
CB0272S-B	20mL x 1 vial	4°C
CB0272S-C	15mL x 1 vial	4°C, protected from light.
CB0272S-D	Powder x 1 vial	Store at 4°C. Fully dissolve the powder with 15mL of distilled water before use, then transfer the entire contents of CB0272S-E into CB0272S-D and mix thoroughly. The mixture can be stored at 4°C for up to one week.
CB0272S-E	Liquid x 1 vial	4°C
CB0272S-Standard	Powder x 1 vial	4°C
<p>Standard Preparation: Prior to use, reconstitute the standard by adding 1mL of Diluent to prepare a 20 µmol/mL phosphate stock solution. This stock solution can be stored at 4°C for up to two weeks.</p> <p>Diluent Preparation: Mix Distilled Water, CB0272S-A, and CB0272S-B at a volume ratio of 10:1:2 (e.g., 10 mL Distilled Water + 1 mL CB0272S-A + 2 mL CB0272S-B). Prepare freshly before each use.</p>		

Note: It is highly recommended to select 2–3 samples with large variations for a preliminary pilot assay before performing the formal measurement.

Instruction for Use

I. Preparation of Lab Instruments

Visible spectrophotometer, 1mL glass cuvettes, analytical balance, vortex mixer/shaker, benchtop centrifuge (room temperature), distilled water, mortar and pestle, 30–50 mesh sieve, etc.

II. Sample Preparation

1. Water Samples: Mix the water sample, CB0272S-A, and CB0272S-B at a volume ratio of 10:1:2 (Recommended: Take 1mL of water sample, add 100µL of CB0272S-A and 200µL of CB0272S-B). Incubate in a boiling water bath (95°C) for 30 min. Cool down to room temperature before assaying.
2. Soil Samples: Air-dry fresh soil samples and pass them through a 30–50 mesh sieve. Mix according to the ratio of Soil Mass (g) : Distilled Water Volume (mL) : CB0272S-A Volume (mL) : CB0272S-B Volume (mL) = 1:10:1:2 (Recommended: Weigh approx. 0.1g of soil sample, add 1mL of distilled water, followed by 100µL of CB0272S-A and 200µL of CB0272S-B). Incubate in a shaking boiling water bath (95°C) for 30 min. Centrifuge at 10,000 × g at room temperature for 10 min, then collect the supernatant for assaying.

III. Measurement Steps

1. Preheat the spectrophotometer for at least 30 min, set the wavelength to 710 nm, and zero the instrument using distilled water.
2. Dilute the standard stock solution (20 µmol/mL) with the prepared Diluent to obtain a series of standard working solutions: 1, 0.75, 0.5, 0.25, 0.125, and 0.0625 µmol/mL.
3. Assay Procedure: Add the following reagents sequentially into 1.5mL EP tubes:

Reagent	Blank Tube (µL)	Sample Tube (µL)	Standard Tube (µL)
Soil Sample		200	
Standard			200
CB0272S-C	200	200	200
CB0272S-D	200	200	200
Distilled Water	600	400	400

Mix thoroughly and incubate at room temperature for 10 min. Transfer the mixture into a 1mL glass cuvette and measure the absorbance at 710 nm. Record the values as Abt, Ast, and Astd.

$$\Delta A = A_{st} - A_{bt}$$

$$\Delta A_{std} = A_{std} - A_{bt}$$

(Note: The blank tube and standard curve only need to be determined 1–2 times per batch).

Note:

Ast: A sample tube

Abt: A blank tube

Astd: A standard tube

IV. Calculations

Standard Curve Plotting: Plot the standard curve with the concentrations of the standard solutions on the x-axis and their corresponding ΔA_{std} on the y-axis to obtain the linear regression equation:

$$y = kx + b$$

Substitute the ΔA of the sample as y into the equation to calculate the concentration x (µmol/mL).

1. Water Samples: Total Phosphate Content (µmol/mL) = $x \times V1 \div (V1 \times W \div V2) = 1.3 x \div W$
2. Soil Samples: Total Phosphate Content (µmol/mL) = $x \times V2 \div V3 = 1.3 x$

Note:

V1: Volume of the sample added to the reaction = 0.2mL

V2: Total volume of the sample = 1.3mL

W: Mass of the soil sample (g)

V3: Actual volume of the original water sample = 1mL

Precautions

1. If the measured absorbance exceeds the linear range of the standard curve, the sample should be appropriately diluted or the sample volume increased before repeating the measurement.
2. This product is intended solely for scientific research use by professional personnel. It is NOT for clinical diagnosis or treatment, NOT for food or drug use, and must NOT be stored in residential areas.
3. For your safety and health, please wear a lab coat and disposable gloves during operation.

TargetMol US

 sales@targetmol.com  (781) 999-4286  www.targetmol.com

 34 Washington Street, Suite 220, Wellesley Hills, MA 02481

TargetMol EU

 sales@targetmol.com  +43(0)676/786025  www.targetmol.com

 Hafenstraße 47-51, 4020 Linz, Austria



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