

## Soil Total Phosphate Assay Kit (MB, Micro Method)

Cat. No.: S1522127 | Pack size: 48 T; 96 T | Storage: 2-8°C; protected from light

### Overview

In acidic solution, under the action of decomposing agent and high temperature, inorganic phosphate and organic phosphate 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, with a characteristic absorbance peak at 710 nm.

### Contents & Storage

| Item No.  | Appearance | Components  | 48 T    | 96 T    | Storage                   |
|-----------|------------|-------------|---------|---------|---------------------------|
| S1522127A | Liquid     | Reagent I   | 10 mL   | 20 mL   | 2-8°C                     |
| S1522127B | Liquid     | Reagent II  | 15 mL   | 30 mL   | 2-8°C                     |
| S1522127C | Liquid     | Reagent III | 2.8 mL  | 5.6 mL  | 2-8°C. Store in the dark. |
| S1522127D | Powder     | Reagent IV  | 1 vial  | 1 vial  | 2-8°C                     |
| S1522127E | Liquid     | Reagent V   | 44.8 µL | 44.8 µL | 2-8°C                     |
| S1522127F | Liquid     | Standard    | 1 mL    | 1 mL    | 2-8°C                     |

Notes: Before formal detection, it is recommended to conduct preliminary trials with 2-3 samples with expected large differences in phosphate content.

### Self-Prepared Consumables and Instruments

1. Microplate reader or visible spectrophotometer (capable of absorbance detection at 710 nm)
2. 96-well microplate or micro glass cuvettes, adjustable pipettes and pipette tips
3. Tabletop centrifuge, 30-50 mesh sieve, thermostatted water bath
4. Deionized water

### Reagent Preparation

**Reagent I:** Ready-to-use; equilibrate to room temperature before use; store at 2-8°C.

**Reagent II:** Ready-to-use; equilibrate to room temperature before use; store at 2-8°C.

**Reagent III:** Ready-to-use; equilibrate to room temperature before use; store at 2-8°C protected from light.

**Working Reagent IV:** Prepare immediately before use. Add 5.6 mL deionized water and dissolve completely, then add Reagent V and mix thoroughly. Working Reagent IV contains a small amount of insoluble precipitate; shake well before use. Stable for 1 week when stored at 2-8°C.

**Reagent V:** Ready-to-use; equilibrate to room temperature before use; store at 2-8°C.

Notes: Reagent I, Reagent II, Reagent III and Reagent V are irritant. Adequate personal protective equipment must be worn during all operations.

**Standard:** Ready-to-use; equilibrate to room temperature before use; store at 2-8°C.

### Standard Solution Preparation

Dilute the 1 µmol/mL standard stepwise as shown in the table below.

| No.   | Standard Volume                    | Deionized Water Volume (µL) | Concentration (µmol/mL) |
|-------|------------------------------------|-----------------------------|-------------------------|
| Std.1 | 50 µL of 100 µL 1 µmol/mL standard | 150                         | 0.25                    |
| Std.2 | 100 µL of Std.1 (0.25 µmol/mL)     | 100                         | 0.125                   |
| Std.3 | 100 µL of Std.2 (0.125 µmol/mL)    | 100                         | 0.063                   |
| Std.4 | 100 µL of Std.3 (0.063 µmol/mL)    | 100                         | 0.031                   |
| Std.5 | 100 µL of Std.4 (0.031 µmol/mL)    | 100                         | 0.016                   |
| Std.6 | 100 µL of Std.5 (0.016 µmol/mL)    | 100                         | 0.008                   |
| Blank | 0                                  | 100                         | 0                       |

Notes: A standard curve must be generated alongside each independent experiment. Diluted standard solutions are unstable and must be used within 4 hours of preparation.

### Sample Preparation

Fresh samples are recommended for immediate detection. If testing cannot be performed promptly, samples can be stored at -80°C for several weeks. Control thawing temperature and duration strictly; complete thawing within 4 hours at room temperature.

1. Air-dry fresh soil samples and sieve through a 30-50 mesh screen.

Mix soil mass (g) : deionized water volume (mL) : Reagent I volume (mL) : Reagent II volume (mL) = 1:10:1:2.

Practical operation: Weigh approximately 0.1 g soil sample, add 1 mL deionized water, followed by 100 µL Reagent I and 200 µL Reagent II. Shake in a 95°C boiling water bath for 30 min, centrifuge at 10,000 g, 25°C for 10 min, collect supernatant for subsequent detection.

## Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for ≥30 min, set detection wavelength to 710 nm. Zero the spectrophotometer with deionized water if applicable.
2. Assay layout (operations performed in 96-well microplate or micro glass cuvettes):

| Reagent            | Blank Well (μL) | Standard Well (μL) | Test Well (μL) |
|--------------------|-----------------|--------------------|----------------|
| Sample             | 0               | 0                  | 40             |
| Standard Solution  | 0               | 40                 | 0              |
| Reagent III        | 40              | 40                 | 40             |
| Working Reagent IV | 40              | 40                 | 40             |
| Deionized Water    | 120             | 80                 | 80             |

3. Mix thoroughly and incubate at 25°C for 10 min. Measure absorbance at 710 nm and record the absorbance values as  $A_{\text{blank}}$ ,  $A_{\text{standard}}$  and  $A_{\text{sample}}$  respectively.

Calculation formulas:  $\Delta A_{\text{sample}} = A_{\text{sample}} - A_{\text{blank}}$ ;  $\Delta A_{\text{standard}} = A_{\text{standard}} - A_{\text{blank}}$ .

Notes: Blank wells and standard curve only need to be tested once per experiment. It is recommended to conduct preliminary experiments with 2-3 samples with obvious expected differences before formal detection.

If  $\Delta A_{\text{sample}}$  is less than 0.01, appropriately increase the sample dosage.

If  $\Delta A_{\text{sample}}$  exceeds the  $\Delta A_{\text{standard}}$  value corresponding to 0.25 μmol/mL, further dilute the sample with deionized water (multiply the final calculation result by the dilution factor), or reduce the amount of soil sample used for extraction.

## Result Calculation

Notes: Two equivalent formulas are provided, namely the full derivation formula and the simplified formula.

### 1. Standard Curve Construction

Plot the standard curve with standard solution concentration as the x-axis and  $\Delta A_{\text{standard}}$  as the y-axis to obtain the standard equation. Substitute  $\Delta A_{\text{sample}}$  into the equation to get x (μmol/mL).

### 2. Calculation of Total Phosphate Content

Total phosphate content (μmol/g) =  $x \times V_{\text{sample}} \div (W \times V_{\text{sample}} \div V_{\text{total-extraction}}) = 1.3 \times x \div W$

$V_{\text{sample}}$ : Volume of sample added, 0.04 mL.

$V_{\text{total-extraction}}$ : Total extraction volume of sample, 1.3 mL.

$W$ : Mass of soil sample, g.

## Results Presentation

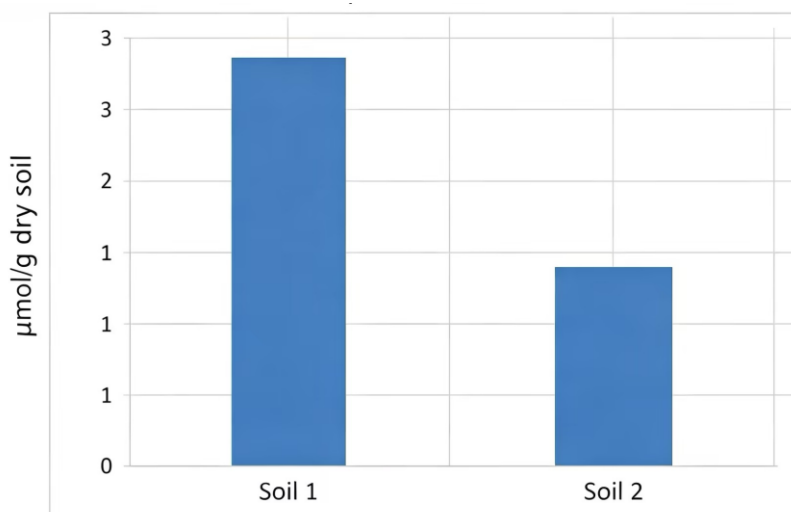


Figure 1. Total phosphate content in soil detected by this kit

## Disclaimer

This product is for research purposes only and is not suitable for clinical diagnosis. Please wear a lab coat and disposable gloves during operation to ensure your safety and health.

## Storage and Shipping

| Item                  | Information                                              |
|-----------------------|----------------------------------------------------------|
| Storage               | Store at 2-8°C, Protected from light                     |
| Shipped In            | Wet ice                                                  |
| Stability And Storage | Store at 2-8°C long term (12 months). Store in the dark. |

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## Limitations & Disclaimer

- ☐ For Research Use Only (RUO). Not for use in human or animal diagnostics, therapeutics, or in vivo applications. Not for food, cosmetic, or household use.
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