

## Soil/Water Total Phosphate Content Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer

**Cat No:** BC4210

**Size:** 50T/48S

### Components:

Reagent I: Concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) 10mL×1 bottle. Self-provided reagent.

Reagent II: Powder×2 bottle, store at 4°C. Add 15mL of distilled water to dissolve before use.

Reagent III: 10mL×1 bottle, store at 4°C and protected from light.

Reagent IV: Powder×1 bottle, store at 4°C and protected from light. Add 10mL of distilled water to dissolve before use. Store at 4°C for one week after dissolution.

Reagent V: Powder×1 bottle, store at 4°C. Add 10mL of distilled water to dissolve before use. Store at 4°C for one week after dissolution.

Reagent VI: 10mL×1 bottle, store at RT.

Standard: Powder×1 tube, store at 4°C. Add 1.4mL of the following dilution solution to dissolve the powder, and make up a 20μmol / mL phosphorus standard solution.

Diluent Preparation: Mix according to the volume of distilled water (mL): reagent I (mL): reagent II (mL) = 10: 1: 2.

Preparation of Phosphorus Reagent: Prepare according to the ratio of H<sub>2</sub>O: reagent IV: reagent V: reagent VI = 2:1:1:1, and the prepared working solution should be light yellow. If the color is changed, the reagent is invalid, if it is blue, it is phosphorus contamination.

Note: It is best to use a new beaker, glass rod and glass pipette for reagent preparation. Disposable plastic utensils can also be used to avoid phosphorus contamination.

### Product Description:

Total phosphate contains various phosphate forms such as orthophosphate, metaphosphate, pyrophosphate, and polyphosphate. It reflects the level of phosphate in water and soil and is an important indicator for evaluating water quality and soil quality.

In an acidic solution, under the conditions of a decomposition agent and high temperature, inorganic phosphate and organic phosphate are hydrolyzed to orthophosphate. Orthophosphate can react with ammonium molybdate to form phosphomolybdic acid, which is reduced to phosphorous molybdenum in the presence of a reducing agent. Blue with characteristic absorption peak at 660nm.

### Required but not Provided Reagents and Equipment:

Spectrophotometer, centrifuge, water bath, scale, transferpette, 1mL glass cuvette, concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), distilled water and 30-50 meshes sieve.

### Sample preparation:

1. Water sample: According to water sample volume (mL): reagent I volume (mL): reagent II

volume (mL) = 10: 1: 2 (recommended to take 1mL of water sample, add 100 $\mu$ L of reagent I and 200 $\mu$ L of reagent II), seal with parafilm. Water bath at 95 $^{\circ}$ C for 30min. After cooling, test.

2. Soil samples: (1) fresh soil samples are air-dried and passed through a 30-50 mesh sieve; (2) according to soil quality (g): distilled water volume (mL): reagent I volume (mL): reagent II volume (mL) = 1: 10: 1: 2 (It is recommended to weigh about 0.1g of soil sample, add 1mL of distilled water, and then add 100 $\mu$ L of reagent I and 200 $\mu$ L of reagent II), and seal with a sealing film. Shake at 95 $^{\circ}$ C in a water bath for 30min, centrifuge at 10,000g for 10min at 25 $^{\circ}$ C, and take the supernatant for measurement.

### Procedure

1. Preheat spectrophotometer for 30 min, adjust wavelength to 660 nm, set zero with distilled water.
2. Dilute the 20  $\mu$ mol / mL standard solution with 1.25, 0.625, 0.3125, 0.15625, 0.078125, 0.039, 0.02  $\mu$ mol / mL standard solution for future use.
3. Sample list: Add the following reagents in 1.5 mL EP tubes:

	Test tube (At)	Standard tube (As)	Blank tube (Ab)
Sample ( $\mu$ L)	100	-	-
Standard solution ( $\mu$ L)	-	100	-
Diluent ( $\mu$ L)	-	-	100
Reagent III ( $\mu$ L)	150	150	150
Phosphorus Reagent ( $\mu$ L)	500	500	500
Distilled water ( $\mu$ L)	250	250	250

Mix well, react at 40 $^{\circ}$ C for 10min, then detect the absorbance at 660nm, named At, As and Ab. Calculate  $\Delta A = A_t - A_b$ ,  $\Delta A_s = A_s - A_b$ . Blank tube, standard tube only need to measure 1-2 times.

### Calculation

1. According to concentration of standard solution and absorbance to create the standard curve, take standard solution as X-axis,  $\Delta A_s$  as Y-axis. Take  $\Delta A$  into the equation to obtain x ( $\mu$ mol/mL)
2. Total phosphate content in soil sample ( $\mu$ mol / g soil sample) =  $x \times V_s \div (V_s \times W \div V_{st}) = 1.3x \div W$   
Total phosphate content in water sample ( $\mu$ mol / mL water sample) =  $x \times V_{st} \div V_w = 1.3x$

$V_s$ : Sample volume, 0.1mL;

$V_{st}$ : the total volume of liquid added to the soil sample during sample processing, 1.3 mL;

$V_w$ : Water sample volume, 1 mL.;

$W$ : Soil sample weight, g.

### Note:

1. Phosphorus reagents should be prepared and used immediately. The normal color is light yellow. If it changes color or turns blue, it will be invalid.
2. After adding reagent III, flocculent precipitation may occur, which has no effect on subsequent experiments. It will be eliminated by itself after adding the phosphorus reagent.
3. If the measured absorbance value exceeds the absorbance value in the linear range, you can increase the sample volume or dilute the sample before performing the measurement.

**Experimental examples:**

1. Take 0.1g of clover soil, add 1mL of distilled water, then add 100 $\mu$ L of Reagent I and 200 $\mu$ L of Reagent II. After centrifugation, take the supernatant and dilute it by 2 times, and then follow the measurement procedure. Calculate  $\Delta A = A_t - A_b = 1.162 - 0.006 = 1.156$ . Bring the result into the standard curve  $y = 0.8377x + 0.0055$ , and calculate  $x = 1.3734$ . The content is calculated according to the sample mass.

Total phosphate content in soil sample ( $\mu\text{mol} / \text{g soil sample}$ ) =  $1.3x \div W \times 2(\text{dilution times}) = 35.71 \mu\text{mol/g soil sample}$ .

2. Take 0.1g of clover soil, add 1mL of distilled water, then add 100 $\mu$ L of Reagent I and 200 $\mu$ L of Reagent II. After centrifugation, take the supernatant and dilute it by 2 times, and then follow the measurement procedure. Calculate  $\Delta A = A_t - A_b = 1.087 - 0.006 = 1.081$ . Bring the result into the standard curve  $y = 0.8377x + 0.0055$ , and calculate  $x = 1.2839$ . The content is calculated according to the sample mass.

Total phosphate content in soil sample ( $\mu\text{mol} / \text{g soil sample}$ ) =  $1.3x \div W \times 2(\text{dilution times}) = 33.38 \mu\text{mol/g soil sample}$ .

**Related products:**

BC2880/BC2885 Soil Phosphate(S-PHOS) Content Assay Kit

BC2890/BC2895 Soil Phosphorus Content Assay Kit

**Technical Specifications:**

Minimum Detection Limit: 0.0065  $\mu\text{mol/mL}$

Linear Range: 0.0098-1.25  $\mu\text{mol/mL}$