

# Soil Total/Organic /Inorganic Phosphorus Content Assay Kit

Note: The reagents have been changed, please be aware of and follow this instruction strictly.

Operation Equipment: Spectrophotometer/Microplate reader

**Cat No:** BC2895 **Size:** 100T/48S

## **Components:**

Reagent I: Liquid 100 mL×1, store at 2-8°C. Reagent II: Liquid 5.5 mL×1, store at 2-8°C.

**Reagent III:** Powder×1, store at 2-8°C. Add 4 mL of distilled water to fully dissolve. Unused reagent is still stored at 2-8°C for four weeks.

**Reagent IV:** Powder×1, store at 2-8°C. Add 4 mL of distilled water to fully dissolve. Unused reagent is still stored at 2-8°C for four weeks.

**Preparation of reagents for phosphorus fixation:** Reagent III, Reagent IV and Reagent II are mixed by the ratio of 1:1:1 to make before use. The prepared phosphorus fixation reagent should be light yellow in color. If it is colorless, the reagent is invalid, if it is blue, it is phosphorus contamination, limit to use on the same day. During the preparation, black solid may be produced, its does not affect the results, be careful not to inhale the black solid when aspirating. (Note: It is best to use new beakers, glass rods and glass pipettes, or disposable plastic containers to avoid phosphorus contamination.

Standard: Liquid 1 mL×1, 10 mmol/L inorganic phosphorus standard, store at 2-8°C

## **Product Description:**

Soil phosphorus includes organic and inorganic phosphorus. the inorganic phosphorus can directly used by plants. Soil organic phosphorus is mineralized and decomposed into inorganic phosphorus. Determine the total phosphorus, organic phosphorus and inorganic phosphorus in the same time can fully reflect the condition of soil phosphorus nutrition.

Molybdenum blue is used to determine phosphorus. One sample of soil is taken and the content of inorganic phosphorus is determined by extraction method. The content of total phosphorus was measured by taking another sample after burning at high temperature. The content of organic phosphorus is calculated by subtracting the content of inorganic phosphorus from the total phosphorus content.

## Required reagents and equipment:

Spectrophotometer/Microplate reader, micro glass cuvette/96 well flat-bottom plate, centrifuge, water bath, scale, transferpettor, 550 °C high temperature electric stove, distilled water and 100 meshes sieve (or smaller).

#### **Procedure:**

I. Sample preparation:

BC2895 -- Page 1/3



- 1. Inorganic phosphorus: Weigh 0.01g of air-dried soil samples through a 30-50 mesh sieve, transfer to a 1mL centrifuge tube, add 1mL of reagent I, shake and mix, and then placed in a 45 °C water bath for 1h, 8000rpm, 25 °C centrifugation for 10min, take the supernatant I, used for the determination of inorganic phosphorus content.
- 2. Total Phosphorus: Take air-dried soil samples through a 100-mesh sieve, burn at 550 °C for 1h, cool down and weigh about 0.01g, transfer to a 1mL centrifuge tube, add 1mL of reagent I, shake and mix well, and then placed in a 45 °C water bath for 1h, 8000rpm, 25 °C, centrifuged for 10min, and the supernatant II was taken and used for the determination of total phosphorus content.

## **II. Determination**

- 1. Preheat spectrophotometer/ microplate reader for 30 min, adjust wavelength to 660 nm, spectrophotometer set zero with distilled water.
- 2. Adjust the temperature of water bath to 40°C.
- 3. Preparation of 1 μmol/mL standard solution: Take 100 μL of 10 μmol/mL phosphorus standard solution, add 900 μL of distilled water, mix well, and prepare 1 μmol/mL standard solution. The standard solution should be ready for use.
- 4. Blank tube: Add  $100\mu$ L of distilled water and  $100\mu$ L of phosphorus fixation to centrifuge tube, incubate at  $40^{\circ}$ C water bath for 10min after mix thoroughly. Detect the absorbance of 660 nm after cooling, record  $A_B$ .
- 5. Standard tube: Add  $10\mu$ L of standard,  $90\mu$ L of distilled water and  $100\mu$ L of phosphorus fixation to centrifuge tube, incubate at  $40^{\circ}$ C water bath for 10min after mix thoroughly. Detect the absorbance of 660 nm after cooling, record  $A_s$ .
- 6. Test tube: Add 10  $\mu$ L of supernatant I or supernatant II, 90 $\mu$ L of distilled water and 100 $\mu$ L of phosphorus fixation to centrifuge tube, incubate at 40°C water bath for 10min after mix thoroughly. Detect the absorbance of 660 nm after cooling, record A<sub>T</sub>.

#### III. Calculation

1. Soil inorganic phosphorus ( $\mu$ mol /g) =[ $C_S \times (A_T - A_B) \div (A_S - A_B)] \times V_T \div W \times 30.97$ =30.97×( $A_T - A_B$ )÷( $A_S - A_B$ )÷W

 $C_S$ : 1 $\mu$ mol/mL;

30.97: relative atomic mass of phosphorus;

W: Soil sample weight, g;

V<sub>T</sub>: Total volume of supernatant, 1 mL.

2. Soil total phosphorus (μmol /g)

 $=[C_S\times(A_T-A_B)\div(A_S-A_B)]\times V_T\div W\times 30.97=30.97\times(A_T-A_B)\div(A_S-A_B)\div W$ 

 $C_S$ : 1 $\mu$ mol/mL;

30.97: relative atomic mass of phosphorus;;

W: Soil sample weight, g;

V<sub>T</sub>: Total volume of supernatant II, 1mL.

3. Soil organic phosphorus ( $\mu$ mol /g) =Soil total phosphorous-Soil inorganic phosphorous.

BC2895 -- Page 2 / 3



## Note:

- 1. If the absorbance value is greater than 1, the sample should be diluted with distilled water.
- 2. The blank tube and standard tube only need to be measured 1-2 times
- 3. The colorimetry should be completed within 40 minutes.

## **Related Products:**

BC2880/BC2885 Soil Phosphate(S-PHOS) Content Assay Kit BC2870/BC2875 Soil Hydrargyrum(S-Hg) Content Assay Kit BC2980/BC2985 Soil Available sulfur Content Assay Kit

## **Technical Specifications:**

The detection limit: 0.3932 µg/mL

Linear range: 1-400 μg/mL



