Neutral/Alkaline Soil Available Phosphorous Content Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate reader

Cat No: BC2965

Size: 100T/96S

Components:

Extract solution: Liquid 125mL×1 bottle, store at 4℃.

Reagent I: Powder×1 bottle, store at 4℃. Dilute with 5 mL of distilled water before use. Unused reagent can be stored for one week at 4 ℃.

Reagent II: Powder×1 bottle, store at 4℃. Dilute with 5 mL of distilled water before use. Unused reagent can be stored for one week at 4 ℃.

Reagent III: Liquid 5 mL×1 bottle, store at room temperature.

Standard: Liquid 1 mL×1, 10 μmol/mL standard phosphorus stock solution, store at 4℃.

Phosphorus fixing reagent: Prepare reagents for determining phosphorus content: make solution as the volume ratio of H_2O: Reagent I: Reagent II: Reagent III =2:1:1:1, which should be light yellow. It shows lose efficacy if color is changed, phosphorus pollution if color is change to blue. Prepare the reagent when it will be use.

Note: It is better to use new beaker, glass rod and glass pipettes, or disposable plastic ware when making reagent to avoid phosphorus pollution.

Product Description:

Rapidly available phosphorus is a phosphorus component that can be absorbed by plants in the soil, including all water-soluble phosphorus, partially adsorbed phosphorus, easily mineralized organic phosphorus, and some dissolved precipitated phosphates.

The alkaline -soluble phosphorus and adsorbed phosphorus are extracted by a weak alkaline method, and molybdenum blue and phosphate generated a substance with a characteristic absorption peak at 660 nm. By measuring the light absorption at 660 nm, the phosphorus content can be calculated.

Required reagents and equipment:

Spectrophotometer/ microplate reader, centrifuge, water bath, analytical balance, transferpettor, micro glass cuvette/ 96 well flat-bottom plate, distilled water, vortex oscillator and 20 meshes sieve.

Procedure:

I. Preparation of sample: Fresh soil samples are naturally air-dried or oven-dried at 37℃, pass through a 20 mesh sieve. Take 0.05 g of air-dried soil sample and add 1 mL of extraction solution. Shake and mix thoroughly, then incubate at 25℃ water bath for 1 h, centrifuge at 10000 g for 10 minutes at room temperature, take supernatant to be tested.

II. Determination procedure:

1. Preheat spectrophotometer/ microplate reader for 30 minutes, adjust wavelength to 660 nm, set

zero with distilled water.

2. Standard: Dilute the 10 μmol/mL standard solution to 3, 2, 1, 0.5, 0.25, 0.125 μmol/mL with extraction solution.

3. Add reagents in 1.5 mL EP tubes as the following:

Reagent (μL)	Test tube (T)	Standard tube (S)	Blank tube (B)
Sample	20	-	-
Standard	-	20	-
Extract solution	40	40	60
Phosphorus fixing reagent	80	80	80
H_2O	60	60	60
Mix thoroughly and standing for 30 minutes at 25℃.Add the mixture into micro glass cuvette/ 96 well flat-bottom plate, and detect the absorbance value of each tube at 660nm and noted as A_T, A_S, A_B. $\Delta A_T=A_T-A_B$, $\Delta A_S=A_S-A_B$. Blank tubes only need to be tested 1-2 times.			

III. Calculation

1. Standard curve.

The concentration of standard solution as x-axis, ΔA_S as y-axis, obtain the equation y=kx+b. Take ΔA_T to the equation to acquire x (μmol/mL) value.

2. Calculation:

Rapidly available phosphorus (μmol/g weight) = $x\times Vs\div(Vs\times W\div V_{ST})=x\div W$

Vs: Sample volume, 0.02 mL;

V_{ST}: Extract solution volume, 1 mL;

W: Soil sample weight, g.

Note:

1. Prepare the phosphorus fixing reagent when it will be used. The normal color of this liquid is light yellow, and it will be invalid if it changes color or turns blue.

2. This method has the characteristics of trace, sensitive and rapid. It is better to use new beaker, glass rod and glass pipettes, or disposable plastic ware when making reagent to avoid phosphorus pollution.

3. Test immediately after color rendering.

4. If the measured absorbance exceeds the linear range, the sample can be determined after being appropriately diluted with Extract solution. When calculation, multiply the calculation formula by the corresponding dilution factor.

5. When testing with 96 well plate, pay attention to whether there are bubbles in the liquid in the plate well. If there are bubbles, remove the bubbles before measuring the OD value.

Technical Specifications:

The detection limit: 0.018 μg/mL

Linear range: 0.03125-3 μg/mL