



## CheKine™ Micro Soil Phosphate (S-PHOS) Assay Kit

Cat #: KTB4011

Size: 48 T/96 T

	<b>Micro Soil Phosphate (S-PHOS) Assay Kit</b>		
<b>REF</b>	<b>Cat #:</b> KTB4011	<b>LOT</b>	<b>Lot #:</b> Refer to product label
	<b>Applicable samples:</b> Soil		
	<b>Storage:</b> Stored at 4°C for 12 months, protected from light		

## Assay Principle

Phosphorus is an essential nutrition for plants growth and development, and exist in the form of organic or inorganic phosphate in soil. Plants mainly import inorganic phosphate from soil through roots. Organic phosphate must be further degraded into inorganic phosphate and absorbed by plants. CheKine™ Micro Soil Phosphate (S-PHOS) Assay Kit was designed for soil inorganic phosphate quantity. In principle, inorganic phosphate was extracted from soil, under acidic conditions, the phosphate content was determined by molybdenum blue, and there is a characteristic absorption peak at 660 nm.

## Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Solution	50 mL	100 mL	4°C
Reagent I	1	1	4°C, protected from light
Reagent II	1	1	4°C, protected from light
Reagent III	2 mL	4 mL	RT
Standard	1 mL	1 mL	4°C

## Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 660 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Analytical balance, Adjustable water bath pot, centrifuge, 100 sieve meshes
- Deionized water

## Reagent Preparation

**Extraction Solution:** Ready to use as supplied; Equilibrate to room temperature before use. Store at 4°C.

**Reagent I** : Add 2 mL deionized water for 48 T and 4 mL deionized water for 96 T to fully dissolve before use, and store it at 4°C, protected from light.

**Reagent II** : Add 2 mL deionized water for 48 T and 4 mL deionized water for 96 T to fully dissolve before use, and store it at 4°C, protected from light.

**Reagent III**: Ready to use as supplied; Store at room temperature.

**Standard**: Ready to use as supplied; Before use, balance to room temperature; Store at 4°C.

**Phosphorus quantitative reagent**: Prepare according to the ratio as Deionized water :Reagent I :Reagent II :Reagent III =2:1:1:1, the color of the prepared phosphorus quantitative reagent should be yellow. The reagent maybe invalid if there is no color, or maybe phosphorus pollution if the color is blue. So, please prepare it depend on your need.

## Sample Preparation

1. Soil samples: Take appropriate amount of air dried soil sample, remove stones, leaves and other impurities, pass through a 100 mesh sieve, accurately weigh 0.01 g, add 1 mL of Extraction Solution, shake and mix at room temperature, and then place it in a water bath at 40°C for 1 h, centrifuge at 8,000 g for 10 min at 25°C, and the supernatant was used for assay.

## Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 660 nm, visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement. (The following are operated in a 1.5 mL centrifuge tube )

Reagent	Blank tube (μL)	Standard tube (μL)	Test tube (μL)
Sample Supernatant	0	0	40
Deionized Water	40	0	0
Phosphorus Quantitative Reagent	200	200	200
Standard	0	40	0

3. Mix well, put it in 40°C water bath for 10 min, cool down at room temperature for 10 min, and then take 200 μL into 96-well plate/microglass cuvette to measure the absorbance at 660 nm. The absorbance of blank tube, standard tube, test tube was recorded as  $A_{Blank}$ ,  $A_{Standard}$  and  $A_{Test}$ . Finally, calculate  $\Delta A_{Test} = A_{Test} - A_{Blank}$ ,  $\Delta A_{Standard} = A_{Standard} - A_{Blank}$ .

**Note: Blank tube and standard tube only need to measure 1 time. In order to guarantee the accuracy of experimental results, pre-experiments were suggested using 2-3 samples with potential significant differences. Increase the sample appropriately. If  $\Delta A_{Test}$  is greater than 1.0, the sample can be appropriately diluted with Extraction Solution, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.**

## Data Analysis

Calculation of inorganic phosphate content in the sample

The calculation formula measured using a 96-well plate/ microglass cuvette as follows:

$$S\text{-PHOS } (\mu\text{mol /g dry weight}) = C_{\text{Standard}} \times \Delta A_{\text{Test}} \div \Delta A_{\text{Standard}} \times V_{\text{Total}} \div W = 0.5 \times \Delta A_{\text{Test}} \div \Delta A_{\text{Standard}} \times V_{\text{Total}} \div W = 50 \times \Delta A_{\text{Test}} \div \Delta A_{\text{Standard}}$$

$C_{\text{Standard}}$ : 500 μmol /L;  $V_{\text{Total}}$ : Total volume of supernatant, 1 mL= 0.001 L; W: Sample weight, g.

## Precautions

1. The phosphorus quantitative reagent needs to be prepared before use, and used within the same day.
2. Finish the measurement within 40 min.

## Typical Data

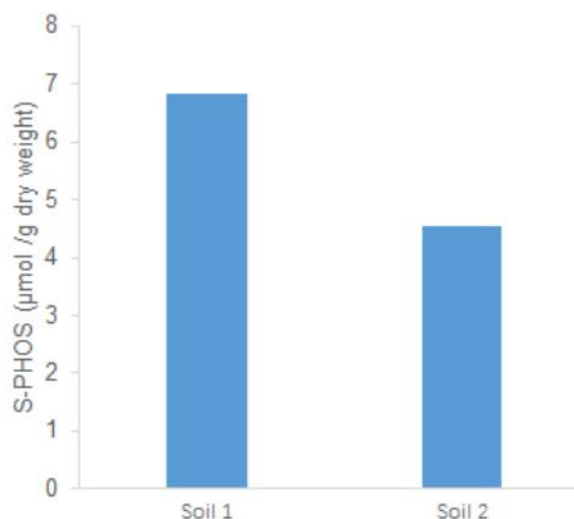


Figure 1. Determination of Inorganic Phosphate Content in Soil

## Recommended Products

Catalog No.	Product Name
KTB3040	CheKine™ Micro Glutamate Synthase (GOGAT) Assay Kit
KTB3041	CheKine™ Micro Glutamic Acid Dehydrogenase (GDH) Assay Kit

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.