



## CheKine™ Micro Soil Total Phosphate Assay Kit

Cat #: KTB4042

Size: 48 T/96 T

	<b>Micro Soil Total Phosphate Assay Kit</b>		
<b>REF</b>	Cat #: KTB4042	<b>LOT</b>	Lot #: Refer to product label
	<b>Applicable sample:</b> Soil samples		
	<b>Storage:</b> Stored at 4°C for 12 months, protected from light		

### Assay Principle

In acidic solution, inorganic phosphate and organic phosphate will be hydrolyzed to orthophosphate under decomposition agent and high temperature conditions. Orthophosphate can react with ammonium molybdate to generate phosphomolybdic acid, which is reduced to phosphomolybdic blue in the presence of reductant, and has a characteristic absorption peak at 710 nm.

### Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent I	10 mL	20 mL	4°C
Reagent II	20 mL	40 mL	4°C
Reagent III	2 mL	4 mL	4°C, protected from light
Reagent IV	1	1	4°C
Reagent V	16 µL	32 µL	4°C
Standard	1 mL	1 mL	4°C

### Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 710 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Centrifuge, 30-50 mesh sieve, thermostat water bath
- Deionized water

### Reagent Preparation

**Reagent I:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

**Reagent II:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

**Reagent III:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

**Reagent IV:** Prepare before use, add 4 mL deionized water to dissolve it fully, and then add Reagent V to mix it fully. Store at 4°C.

**Reagent V:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

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

**Standard preparation:** Use 1 µmol/mL standard, prepare standard curve dilution as described in the table.

Num.	Standard Volume	Extraction Buffer Volume (µL)	Concentration (µmol/mL)
Std.1	50 µL of 100 µL 1 µmol/mL	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

## Sample Preparation

**Note:** Fresh samples are recommended. If the experiment is not carried out immediately, the samples can be stored at -80°C for several weeks. During the determination, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Fresh soil sample is air dried, sieved through 30-50 mesh sieve. According to the soil sample mass (g): deionized water volume (mL): Reagent I volume (mL): Reagent II volume (mL) = 1:10:1:2 (it is recommended to weigh about 0.1 g of soil sample, add 1 mL of deionized water, and then add 100 µL Reagent I and 200 µL Reagent II), 95°C boiling water bath shaking for 30 min, centrifuge at 10,000 g for 10 min at 25°C, take the supernatant to be tested.

## Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 710 nm, visible spectrophotometer was returned to zero with deionized water.

2. Assay procedure (The following were operated in the 96-well plate or microglass cuvette):

Reagent	Blank Well (µL)	Standard Well (µL)	Test Well (µL)
Supernatant	0	0	40
Standard	0	40	0
Reagent III	40	40	40
Reagent IV	40	40	40
Deionized Water	120	80	80

3. After mixing, keep at 25°C for 10 min, and measure the absorbance at 710 nm. The absorbance of blank well, standard well, test well recorded as  $A_{\text{Blank}}$ ,  $A_{\text{Standard}}$  and  $A_{\text{Test}}$ . Finally, calculate  $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Blank}}$ ,  $\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$ .

**Note:** Blank well and standard well only need to measure 1 time. In order to guarantee the accuracy of experimental results, pre-experiments are suggested use 2-3 samples with potential significant difference. If  $\Delta A_{\text{Test}}$  is greater than 0.5, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

## Data Analysis

**Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.**

1. Drawing of standard curve:

With the concentration of the standard solution as the x-axis and the  $\Delta A_{\text{Standard}}$  as the y-axis, draw the standard curve, get the standard equation  $y=kx+b$ , and bring the  $\Delta A_{\text{Test}}$  into the equation to get the x value ( $\mu\text{mol/mL}$ ).

3. Calculation of total phosphate content:

Total phosphate content ( $\mu\text{mol/g}$ )= $x \times V_{\text{Sample}} \div (W \times V_{\text{Sample}} \div V_{\text{Total sample}})$  =  **$1.3 \times x \div W$**

$V_{\text{Sample}}$ : added sample volume, 0.04 mL;  $V_{\text{Total sample}}$ : total sample volume, 1.3 mL;  $W$ : weight of soil sample, g.

## Typical Data

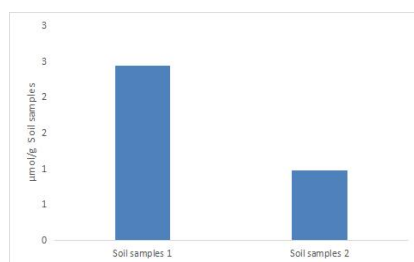


Figure 1. Determination total phosphate in soils by this assay kit

## Precautions

1. The prepared Reagent IV contains a small amount of insoluble matter, which should be shaken up before use and used up within a week.

## Recommended Products

Catalog No.	Product Name
KTB4011	CheKine™ Micro Soil Phosphate (S-PHOS) Assay Kit
KTB4013	CheKine™ Micro Neutral/Alkaline Soil Available Phosphorous Assay Kit

## Disclaimer

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