



## CheKine™ Micro Soil Neutral Phosphatase (S-NP) Activity Assay Kit

Cat #: KTB4043

Size: 48 T/96 T

	<b>Micro Soil Neutral Phosphatase (S-NP) Activity Assay Kit</b>		
<b>REF</b>	<b>Cat #:</b> KTB4043	<b>LOT</b>	<b>Lot #:</b> Refer to product label
	<b>Applicable:</b> Soil sample		
	<b>Storage:</b> Stored at 4°C for 6 months, protected from light		

### Assay Principle

Soil phosphatase is a kind of enzyme that catalyzes the mineralization of soil organic phosphorus. Its activity directly affects the decomposition and transformation of organic phosphorus in soil and its bioavailability. It is an index to evaluate the direction and intensity of soil phosphorus biotransformation. Soil phosphatase is significantly affected by soil carbon content, nitrogen content, available phosphorus content and pH. According to the optimal pH range, it is generally divided into three types: acidic, neutral and alkaline. Soil neutral phosphatase (S-NP) can catalyze the hydrolysis of disodium phenylphosphate to produce phenol and disodium hydrogen phosphate in a neutral environment. The activity of soil neutral phosphatase can be characterized by measuring the amount of phenol produced.

### Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent I	21 mL	42 mL	4°C, protected from light
Reagent II	1	1	4°C
Reagent III	1.25 mL	2.5 mL	4°C
Reagent IV	1	1	4°C
Standard	1 mL	1 mL	4°C, protected from light

### 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
- Constant temperature shaker, ice maker, centrifuge, 30-50 mesh sieve
- Deionized water, toluene, anhydrous ethanol

## Reagent Preparation

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

**Reagent II:** Prepared before use. add 50 mL deionized water for 48 T and 100 mL deionized water for 96 T to fully dissolve. Store at 4°C.

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

**Reagent IV:** Prepared before use. add 288 µL anhydrous ethanol, 12 µL deionized water for 48 T and 576 µL anhydrous ethanol, 24 µL deionized water for 96 T to fully dissolve. Store at 4°C, protected from light.

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

## 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 samples naturally air dried or air dried in an oven at 37°C and sieved through 30-50 mesh sieve. Weigh 0.1g air dried soil sample into 1.5 mL EP tube and add 50 µL toluene, gently shake for 15 min to mix it.
2. Add 0.4 mL Reagent I, mix well, place it in a 37°C constant temperature shaker and start timing, and catalyze the reaction for 24 h; When the time comes, quickly add 1 mL of Reagent II and mix well to stop the enzyme catalyzed reaction.
3. Centrifuge at 8,000 g for 10 min at 25°C, and the supernatant was placed on ice for testing.

## 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. Operation table (The following were operated in the 96-well plate or microglass cuvette):

Reagent	Test Well (µL)	Blank Well (µL)	Standard Well (µL)
Supernatant	10	0	0
Standard	0	0	10
Deionized Water	0	10	0
Reagent III	20	20	20
Reagent IV	4	4	4
Deionized Water	4	4	4

Mix well and add deionized water immediately after color development

Deionized Water	166	166	166
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3. After mixing, keep at 25°C for 30 min, and measure the absorbance at 660 nm, record it as  $A_{\text{Test}}$ ,  $A_{\text{Blank}}$  and  $A_{\text{Standard}}$ .

**Note: Blank well and standard well only need to be measured once, in order to guarantee the accuracy of experimental results, pre-experiments are suggested use 2-3 samples with potential significant difference, and the sample size to be measured can be appropriately adjusted according to the test results.**

## 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.**

S-NP activity calculation formula:

Definition of active unit: 1  $\mu\text{mol}$  phenol released per g of soil sample per day at 37°C is defined as a unit of enzyme activity.

$$\text{S-NP (U/g soil sample)} = \frac{C_{\text{Standard}} \times (A_{\text{Test}} - A_{\text{Blank}}) \div (A_{\text{Standard}} - A_{\text{Blank}})] \times V_{\text{Total}} \div W \div T = 0.725 \times (A_{\text{Test}} - A_{\text{Blank}}) \div (A_{\text{Standard}} - A_{\text{Blank}}) \div W$$

$C_{\text{Standard}}$ : 0.5  $\mu\text{mol/mL}$ ;  $V_{\text{Total}}$ : total volume of catalytic system, 1.45 mL; W: weight of soil sample, 0.1 g; T: catalytic reaction time, 24 h=1 d.

## Typical Data

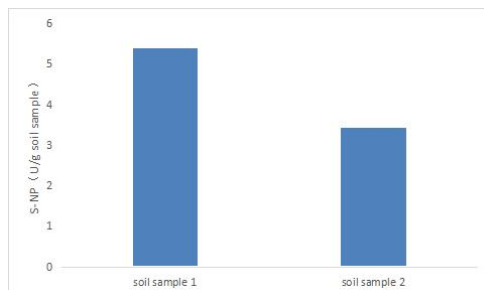


Figure 1. Determination of neutral phosphatase activity in soil by this assay kit

## Precautions

1. If Reagent IV turns brown, it cannot be used.

## Recommended Products

Catalog No.	Product Name
KTB4010	CheKine™ Micro Soil Nitrate Reductase (S-NR) Activity Assay Kit
KTB4020	CheKine™ Micro Soil Leucine Arylamidase (S-LAP) Activity Assay Kit

## Disclaimer

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