



## CheKine™ Micro Soil Ammonium Nitrogen Assay Kit

Cat #: KTB4015

Size: 96 T/96 S

	<b>Micro Soil Ammonium Nitrogen Assay Kit</b>		
<b>REF</b>	Cat #: KTB4015	<b>LOT</b>	Lot #: Refer to product label
	<b>Applicable sample:</b> Soil sample		
	<b>Storage:</b> Stored at 4°C for 12 months, protected from light		

### Assay Principle

Soil ammonium nitrogen is an important component of soil available nitrogen, which can be directly absorbed and utilized by plants. Ammonium nitrogen in soil reacts with hypochlorite and phenol in strong alkaline medium to produce water-soluble dye indophenol blue, which has a characteristic absorption peak at 625 nm, and the absorbance value is directly proportional to the content of ammonium nitrogen.

### Materials Supplied and Storage Conditions

Kit components	Size (96 T)	Storage conditions
Extraction Buffer	70 mL×2	4°C
Reagent I	Powder×2 vials	4°C, protected from light
Reagent II	11.2 mL	4°C, protected from light
Reagent III	2.8 mL	4°C
Standard	1 mL	4°C

**Note:** Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

### Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 625 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Thermostatic shaker, centrifuge
- Deionized water

### Reagent Preparation

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

**Working Reagent I:** Prepare before use, add 5.6 mL deionized water to each tube according to the dosage, and use after fully

dissolving. The remaining reagent can be stored at 4°C, protected from light for 2 weeks.

**Note: Reagent I is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.**

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

**Reagent III:** 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 500 µmol/mL standard, prepare standard curve dilution as described in the table.

Num.	Standard Volume	Extraction Buffer Volume (µL)	Concentration (µmol/mL)
Std.1	5 µL 500 µmol/mL	995	2.5
Std.2	100 µL of Std.1 (2.5 µmol/mL)	100	1.25
Std.3	100 µL of Std.2 (1.25 µmol/mL)	100	0.625
Std.4	100 µL of Std.3 (0.625 µmol/mL)	100	0.313
Std.5	100 µL of Std.4 (0.313 µmol/mL)	100	0.156
Std.6	100 µL of Std.5 (0.156 µmol/mL)	100	0.078
Std.7	100 µL of Std.6 (0.078 µmol/mL)	100	0.039
Blank	0	100	0

**Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.**

## Sample Preparation

**Note: Air dry or oven dry of soil can cause remarkable changes in nitrate nitrogen content, so it is recommended to use fresh soil or soil samples with same treatment for measurement. 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.**

Add Extraction Buffer according to the ratio of soil mass (g): Extraction Buffer volume (mL) of 1:5-10 (It is recommended to weigh about 0.1 g soil sample and add 1 mL of Extraction Buffer volume). Shake and extract in thermostatic shaker at 37°C for 1 h centrifuge at 10,000 g for 10 min at 25°C, and keep the supernatant for test.

## Assay Procedure

- Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 625 nm, visible spectrophotometer was returned to zero with deionized water.
- Operation table (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	20
Extraction Buffer	20	0	0
Standards	0	20	0
Working Reagent I	80	80	80
Reagent II	80	80	80

Mix thoroughly and keep at 25°C for 1 h

Reagent III	20	20	20
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- After thorough mixing, measure the absorbance value at 625 nm immediately, record it as  $A_{\text{Blank}}$ ,  $A_{\text{Standard}}$  and  $A_{\text{Test}}$ . Calculate

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$$\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Blank}}, \Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}.$$

**Note:** Blank well and standard well only need to measure 1 time. In order to guarantee the accuracy of experimental results, pre-experiment for 2-3 samples with potential significant difference was recommended. If  $\Delta A_{\text{Test}}$  is less than 0.02, increase the sample quantity appropriately. If  $\Delta A_{\text{Test}}$  is larger than 2.5  $\mu\text{mol/mL}$  of  $\Delta A_{\text{Standard}}$ , the sample can be appropriately diluted with Extraction Buffer, 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 ( $\mu\text{mol/mL}$ ).

2. Calculation of ammonium nitrogen content:

Ammonium nitrogen content ( $\mu\text{g/g}$  soil sample) =  $x \times V_{\text{Total sample}} \times 18 \div W = \mathbf{180 \times x}$

$V_{\text{Total sample}}$ : added Extraction Buffer volume, 1 mL; W: sample weight, 0.1 g; 18: molar mass of  $\text{NH}_4^+$ ,  $\mu\text{g}/\mu\text{mol}$ .

## Typical Data

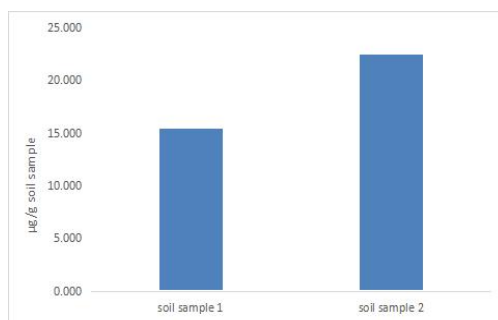


Figure 1. Determination ammonium nitrogen in soils by this assay kit

## Precautions

1. If you want to compare the ammonium nitrogen content between different samples, you need to maintain the same sampling depth, dry the soil sample, and compare it after calculating according to the dry weight.
2. Protective measures shall be taken during operation.

## Recommended Products

Catalog No.	Product Name
KTB4011	CheKine™ Micro Soil Phosphate (S-PHOS) Assay Kit
KTB4012	CheKine™ Micro Soil Nitrate Nitrogen Assay Kit

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.