



## CheKine™ Micro Soil Nitrate Nitrogen Assay Kit

Cat #: KTB4012

Size: 48 T/96 T

	<b>Micro Soil Nitrate Nitrogen Assay Kit</b>		
<b>REF</b>	<b>Cat #:</b> KTB4012	<b>LOT</b>	<b>Lot #:</b> 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

Nitrate nitrogen refers to the nitrogen element contained in nitrates. Organic matter in soil decomposes into ammonium salts, which are oxidized and converted into nitrate nitrogen. Under concentrated acid conditions,  $\text{NO}_3^-$  reacts with salicylic acid to produce nitrosalicylic acid. Under alkaline conditions ( $\text{pH} > 12$ ), nitrosalicylic acid turns yellow, and absorbance changes reflect the concentration of  $\text{NO}_3^-$  within a certain range. Thus the nitrate nitrogen content can be calculated by colorimetric measurement.

### Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent I	1	1×2	4°C, protected from light
Reagent II	25 mL	50 mL	4°C
Standard	1	1	4°C

### Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 410 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Analytical balance, thermostatic water bath, thermostatic shaker, centrifuge
- Deionized water, sulfuric acid

### Reagent Preparation

**Reagent I:** Prepare before use, add 1 mL sulfuric acid to each tube according to the dosage and fully dissolve before use; Store at 4°C and protected from light.

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

**Standard:** Prepare before use, add 1 mL deionized water to the tube to prepare 10 mg/mL of NO<sub>3</sub><sup>-</sup> standard solution, take 100 µL of the above solution in a 1.5 mL EP tube, add 900 µL deionized water, which is 1 mg/mL of NO<sub>3</sub><sup>-</sup> standard solution; Store at 4°C.

## Sample Preparation

According to the ratio (1:5-10) of soil sample (g): deionized water (mL) (Generally, it is recommended to weigh about 0.1 g and add 1 mL of deionized water), deionized water was added. 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.

**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.**

## Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 410 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (the following operations are performed in a 1.5 mL centrifuge tube):

Reagent	Blank Well (µL)	Standard Well (µL)	Test Well (µL)
Supernatant	0	0	10
Deionized Water	10	0	0
Standard	0	10	0
Reagent I	20	20	20
Mix thoroughly and keep at 25°C for 30 min			
Reagent II	475	475	475

3. Mix well, vortex to fully dissolve the precipitate, take 200 µL to microglass cuvette or 96 well plate, and measure the absorbance at 410 nm. The absorbance of blank well, standard well, test well was recorded as A<sub>Blank</sub>, A<sub>Standard</sub> and A<sub>Test</sub>. Finally, calculate  $\Delta A_{Test} = A_{Test} - A_{Blank}$ ,  $\Delta A_{Standard} = A_{Standard} - A_{Blank}$ .

**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.**

## 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.**

$$NO_3^- \text{ content } (\mu\text{g/g sample}) = \Delta A_{Test} \div (\Delta A_{Standard} \div C_{Standard}) \times V_{Total \text{ sample}} \div W = 1,000 \times \Delta A_{Test} \div \Delta A_{Standard} \div W$$

W: sample weight, g; C<sub>Standard</sub>: concentration of standard solution, 1,000 µg/mL; V<sub>Total sample</sub>: volume of extraction solution, 1 mL.

## Typical Data

Take 0.1 g of garden soil and add 1 mL deionized water to shake and extract for 1 h. Keep supernatant following the measurement steps. Use a 96 well plate to measure and calculate  $\Delta A_{Test} = A_{Test} - A_{Blank} = 0.062 - 0.047 = 0.015$ ,  $\Delta A_{Standard} = A_{Standard} - A_{Blank} = 1.515 - 0.047 = 1.468$ , calculated as NO<sub>3</sub><sup>-</sup> content based on soil weight:

$$NO_3^- \text{ content } (\mu\text{g/g soil sample}) = 1,000 \times \Delta A_{Test} \div \Delta A_{Standard} \div W = 1,000 \times 0.015 \div 1.468 \div 0.1 = 102.18 (\mu\text{g/g soil sample}).$$

## Precautions

1. Prepare Reagent I before use, and should be used as soon as possible.
2. Reagent I and Reagent II possess corrosion properties, proper protective measures were needed.
3. Soil colloids do not adsorb nitrate ions and  $\text{NO}_3^-$  are easily soluble in water, moving within the soil. Therefore, when measuring multiple or duplicate samples, it is important to maintain the same sampling depth. It is recommended to take samples about 10 cm below the soil surface. The content of different samples is related to the region.

## Recommended Products

Catalog No.	Product Name
KTB4011	CheKine™ Micro Soil Phosphate(S-PHOS) Assay Kit
KTB3080	CheKine™ Micro Plant Nitrate Nitrogen Assay Kit

## Disclaimer

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