

## Soil Nitrate Nitrogen Content Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer/Microplate reader

**Cat No:** BC0045

**Size:** 100T/96S

### Components:

**Reagent I:** Powder×2, store at 2-8°C and protect from light. Add 1 mL of concentrated H<sub>2</sub>SO<sub>4</sub> before use, fully dissolve it. It could be stored at 2-8°C for one week.

**Reagent II:** Liquid 40 mL×1, store at 2-8°C.

**Standard:** Powder×1, store at 2-8°C. 10 mg of potassium nitrate. Dissolve it with 1.386 mL of distilled water to form a NO<sub>3</sub><sup>-</sup>-N standard solution of 1000 µg/mL. It could be stored at 2-8°C for two weeks.

### Product Description:

Nitrate nitrogen is the nitrogen element contained in nitrate. Nitrate nitrogen in soil is one of the main forms of nitrogen absorption by higher plants, and its content is directly related to the yield and quality of crops.

Under the condition of concentrated acid, NO<sub>3</sub><sup>-</sup> reacts with salicylic acid to form nitrosalicylic acid, which is yellow under the condition of alkaline (pH>12), and its color is directly proportional to the content, so the content of nitrate nitrogen can be calculated.

### Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, table centrifuge, water-bath, micro glass cuvette/96 well flat-bottom plate, transferpettor, oscillator and distilled water, concentrated H<sub>2</sub>SO<sub>4</sub>.

### Procedure

#### I. Sample preparation:

Add distilled water according to the ratio of soil mass(g): the volume of distilled water(mL)=1:5 ~ 10 (it is recommended to weigh about 0.1 g of soil sample, add 1 mL of distilled water), shake it in the shaker for one hour. Centrifuge it at 10000 ×g for 10 minutes 25°C and take the supernatant for test (The requirements for selecting soil samples can refer to Note 1-3).

#### II. Determination procedure:

1. Preheat spectrophotometer/ microplate reader for 30 minutes, adjust wavelength to 410 nm, set spectrophotometer to zero with distilled water.
2. Dilute the 1000 µg/mL NO<sub>3</sub><sup>-</sup>-N standard solution 40 times with distilled water to 25 µg/mL standard solution.
3. Operation table:

Reagent (µL)	Test tube (T)	Standard tube (S)	Blank tube (B)
Sample	8	-	-

Standard solution	-	8	-
Distilled water	-	-	8
Reagent I	12	12	12
Mix well and let stand at 25°C for 30 minutes.			
Reagent II	280	280	280
Mix well, vortex and shake to make the precipitate fully dissolved. Take 0.2 mL of the reaction solution to micro glass cuvette/96 well flat-bottom plate, and measure the absorbance at 410 nm, calculate $\Delta A = A_T - A_B$ , $\Delta A_S = A_S - A_B$ . Standard tube and blank tube only need to be measured 1-2 times.			

### III. Calculation

$$\text{NO}_3\text{-N content } (\mu\text{g/g weight}) = \Delta A \div (\Delta A_S \div C_S) \times V_E \div W = 25 \times \Delta A \div \Delta A_S \div W$$

W: Sample mass, g;

C<sub>S</sub>: Concentration of standard solution, 25 μg/mL;

V<sub>E</sub>: Volume of extract solution, 1 mL.

#### Note:

1. Soil colloid does not adsorb nitrate ion, and it is soluble in water and moves inside the soil. Therefore, the same sampling depth should be kept for multiple samples or repeated samples.
2. It is suggested that fresh soil should be used to determine the content of nitrate nitrogen. The samples should be sealed, transported and stored at 4°C, and analyzed within 3 days. Otherwise, it should be stored in small pieces at -20°C (deep freezing), and the nitrate nitrogen in the sample can be stored for several weeks. The temperature and time of thawing should be controlled when measuring the nitrate nitrogen content of deep freezing. When thawing at room temperature, the sample should be thawed, homogenized and extracted within 4 h; if thawing at 4°C, the thawing time should not exceed 48 h.
3. If we want to compare the content of nitrate nitrogen between different samples, we need to dry the soil samples and calculate them according to the dry weight.
4. Both Reagent I and Reagent II are highly corrosive, so protective measures should be taken during operation.
5. If the absorbance value is greater than 1.5, it is recommended to dilute the sample with distilled water before determination.

#### Experimental examples:

1. 0.1 g of soil sample is added with 1 mL of distilled water for shaking extraction for 1 h. After centrifugation, the supernatant is extracted by 96 well plate according to the determination steps. The calculated results are as follows:  $\Delta A = A_T - A_B = 0.161 - 0.049 = 0.112$ ,  $\Delta A_S = 0.296 - 0.049 = 0.247$ . The result is calculated according to sample weight:

$\text{NO}^3\text{-N}$  content ( $\mu\text{g/g}$  weight) =  $25 \times \Delta A \div \Delta A_S \div W = 25 \times 0.112 \div 0.247 \div 0.1 = 113.36 \mu\text{g/g}$  weight.

2. 0.1 g of forest soil is added with 1 mL of distilled water for shaking extraction for 1 h. After centrifugation, the supernatant is extracted by 96 well plate and operated according to the determination steps.  $\Delta A = A_T - A_B = 0.132 - 0.049 = 0.083$ ,  $\Delta A_S = 0.296 - 0.049 = 0.247$ . The result is calculated according to sample weight:

$\text{NO}^3\text{-N}$  content ( $\mu\text{g/g}$  weight) =  $25 \times \Delta A \div \Delta A_S \div W = 25 \times 0.083 \div 0.247 \div 0.1 = 84 \mu\text{g/g}$  weight.

### Recent product citations:

- [1] Yan JF, Xiang L, Zhang BY, Tang C, Xie YQ, Li YW, Feng NX, Liu BL, Li H, Cai QY, Li QX, Zhao HM, Mo CH. Mechanism and Association between Microbial Nitrogen Transformation in Rhizosphere and Accumulation of Ciprofloxacin in Choysum (*Brassica parachinensis*). *Environ Sci Technol*. 2023 Oct 24;57(42):16053-16064. doi: 10.1021/acs.est.3c04709. Epub 2023 Oct 12. PMID: 37824517.
- [2] Wang M, Qi X, Shi Y, Zhao J, Ahmad S, Akhtar K, Chen B, Lian T, He B, Wen R. Sugarcane straw returning is an approaching technique for the improvement of rhizosphere soil functionality, microbial community, and yield of different sugarcane cultivars. *Front Microbiol*. 2023 Mar 14; 14:1133973. doi: 10.3389/fmicb.2023.1133973. PMID: 36998394; PMCID: PMC10043380.

### References:

- [1] Vendrell P F, Zupancic J. Determination of soil nitrate by transnitration of salicylic acid[J]. *Communications in Soil Science and Plant Analysis*, 1990, 21(13-16): 1705-1713.
- [2] Cataldo D A, Maroon M, Schrader L E, et al. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid[J]. *Communications in soil science and plant analysis*, 1975, 6(1): 71-80.

### Related products:

BC2890/BC2895 Total Phosphorus/Organic Phosphorus And Inorganic Phosphorus In Soil Assay Kit  
BC3020/BC3025 Soil Available Boron Assay Kit

### Technical Specifications:

Minimum Detection Limit: 0.2155  $\mu\text{g/mL}$

Linear Range: 0.78125-200  $\mu\text{g/mL}$