

## Plant Nitrate Nitrogen Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Catalog Number:** AK0428

**Size:**50T/24S

### Components:

Reagent I: powder×2 bottle, storage at 4°C protected from light. Add 2 mL concentrated sulfuric acid to each bottle according to dosage before use.

Reagent II: liquid 100 mL×1 bottle, storage at 4°C .

Standard: powder×1 bottle, storage at 4°C, 10 mg KNO<sub>3</sub>. Dissolve thoroughly with 0.935 mL distilled water before use to make 1400 µg/mL NO<sub>3</sub>-N standard solution.

### Product Description:

Nitrate is one of the nitrogen - containing substances absorbed by plants. Nitrate is reduced in roots , branches or leaves, depending on plant type and environmental conditions. Detecting nitrate nitrogen content in plants is significant to understand the nitrogen metabolism mechanism.

NO<sub>3</sub><sup>-</sup> can react with salicylic acid to form nitrosalicylic acid under the condition of concentrated acid, which shows yellow under the condition of pH>12. Within a certain range, the color depth is proportional to the content.

### Reagents and Equipments Required but Not Provided:

Spectrophotometer, water bath, centrifuge, transferpettor, 1 mL glass cuvette, mortar/homogenizer, ice and distilled water.

### Sample preparation:

Add 1 mL of distilled water into 0.1 g of tissue, fully grind at RT and put it in 90°C water bath for 30 min, shaking during the bath. Or put in 90°C shaker, centrifuge at 12000 g, 25°C for 15 min after cooling. Take the supernatant on ice for test.

### Procedure:

1. Preheat spectrophotometer for 30 min, adjust the wavelength to 410 nm, set the counter to zero with distilled water.
2. Dilute 1400 µg/mL NO<sub>3</sub>-N standard solution with distilled water to 28 µg/mL for use.
3. Add the following reagents:

Reagent (µL)	Blank tube A2	Standard tube A1	Test tube A3	Control tube A4
Sample			40	40
Standard		40		
Distilled water	40			60
Reagent I	60	60	60	
Mix thoroughly, stand at 25°C for 30 min.				
Reagent II	1400	1400	1400	1400
Mix thoroughly, shaking until the sediment dissolved thoroughly, take 1 mL from 1 mL glass cuvette, detect absorbance at 410 nm, $\Delta A(\text{standard}) = \Delta A(S) = A1 - A2$ , $\Delta A(\text{test}) = \Delta A(T) = A3 - A4$ .				

### Calculation:

#### 1. Sample weight:

$$\text{NO}_3\text{-N } (\mu\text{g/g weight}) = \Delta A(T) \div (\Delta A(S) \div C) \times V_e \div W = 28 \times \Delta A(T) \div \Delta A(S) \div W$$

#### 2. Protein concentration:

$$\text{NO}_3\text{-N } (\mu\text{g/mg prot}) = \Delta A(T) \div (\Delta A(S) \div C) \times V_e \div (C_{pr} \times V_e) = 28 \times \Delta A(T) \div \Delta A(S) \div C_{pr}$$

C: Standard concentration, 28 µg/mL

C<sub>pr</sub>: Sample concentration (mg/mL);

W: Sample weight (g);

V<sub>e</sub>: Extraction volume, 1 mL;

### Note:

1. Use Reagent I as soon as possible, storage at 4°C for one week;

- Both Reagent I and Reagent II are highly corrosive, and protective measures must be taken during operation.
- If  $\Delta A(T) > 1$ , dilute the sample before the determination.

### Technical Specifications:

Minimum Detection Limit: 0.7534 ug/mL

Linear Range : 0.875-84 ug/mL

### References:

[1] Fuyuan Zhu, Moxian Chen, Wailung Chan, et al. SWATH-MS quantitative proteomic investigation of nitrogen starvation in Arabidopsis reveals new aspects of plant nitrogen stress responses. Journal of Proteomics. September 2018;(IF3.537)

### Related products:

AK0301/AK0300 Nitrate Reductase(NR) Activity Assay Kit

AK0436/AK0435 Glutaminase(GLS) Activity Assay Kit

AK0434/AK0433 Glutamic Acid Dehydrogenase(GDH) Activity Assay Kit

### Experimental example:

- Take 0.1g apple to 1ml distilled water, operate as the procedure after taking the supernatant, test and calculate  $\Delta A(\text{test}) = \Delta A(T) = A3 - A4 = 0.560 - 0.002 = 0.558$ ,  $\Delta A(\text{standard}) = \Delta A(S) = A1 - A2 = 0.563 - 0.01 = 0.553$ , calculate content by sample weight:  $\text{NO}_3\text{-N} (\mu\text{g/g weight}) = 28 * \Delta A \div \Delta A(S) \div W = 28 \times 0.558 \div 0.553 \div 0.1 = 282.5 \mu\text{g/g weight}$ .
- Take 0.1g leaf to 1ml distilled water, operate as the procedure after taking the supernatant, test and calculate  $\Delta A(\text{test}) = \Delta A(T) = A3 - A4 = 0.907 - 0.645 = 0.262$ ,  $\Delta A(\text{standard}) = \Delta A(S) = A1 - A2 = 0.563 - 0.01 = 0.553$ , calculate content by sample weight:  $\text{NO}_3\text{-N} (\mu\text{g/g weight}) = 28 * \Delta A \div \Delta A(S) \div W = 28 \times 0.262 \div 0.553 \div 0.1 = 132.7 \mu\text{g/g weight}$ .