

## Plant Ammonium Nitrogen Content Assay Kit

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

**Operation equipment:** Spectrophotometer

**Cat No:** BC6040

**Size:** 50T/48S

**Product Composition:** Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8°C
Reagent IA	Liquid 5 mL×1	2-8°C
Reagent IB	Liquid 20 mL×1	2-8°C
Reagent II	Liquid 25mL×1	2-8°C
Standard	Liquid 1 mL×1	2-8°C

### Solution Preparation:

1. Reagent I: Before use, according to the sample size, the ratio of Reagent IA : Reagent IB = 0.4mL : 1.6mL ( 2mL, about 5T ) was prepared and used;
2. Standard: 100 $\mu$ mol/mL NH<sub>4</sub><sup>+</sup>;
3. 0.5 $\mu$ mol/mL NH<sub>4</sub><sup>+</sup>: Before use, add 100 $\mu$ L of 100 $\mu$ mol/mL NH<sub>4</sub><sup>+</sup> to 900 $\mu$ L of distilled water to get 10 $\mu$ mol/mL NH<sub>4</sub><sup>+</sup>, then add 50 $\mu$ L of 10 $\mu$ mol/mL NH<sub>4</sub><sup>+</sup> to 950 $\mu$ L of distilled water to get 0.5 $\mu$ mol/mL NH<sub>4</sub><sup>+</sup>, 0.5 $\mu$ mol/mL NH<sub>4</sub><sup>+</sup> was used as the standard tube.

### Description:

As one of the main nitrogen sources for plant absorption and utilization, ammonium nitrogen synthesizes various important biological molecules including amino acids, participates in the nitrogen metabolism process of plants, and has a very important impact on plant growth and crop yield.

According to the reaction principle of ammonium nitrogen and indophenol blue, this method uses phenol-hypochlorite direct chromogenic method to determine ammonium nitrogen. The generated blue indophenol is proportional to the concentration of ammonium nitrogen, and has a special absorption peak at 630 nm. According to this, the content of ammonium nitrogen in the sample can be calculated from the absorption value.

### Reagents and Equipment Required but Not Provided:

Spectrophotometer, water-bath/constant temperature incubator, tabletop centrifuge, adjustable pipette, 1 mL glass cuvette, mortar/homogenizer, distilled water and EP tube.

### Operation procedure:

#### I. Sample preparation

Add extract solution according to the ratio of tissue mass (g) : extract solution (mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0mL extract solution), after ice bath homogenization, centrifuge at room temperature, 10000rpm for 10min, take supernatant for test.

## II. Determination procedure

1. Preheat spectrophotometer for more than 30 minutes, adjust wavelength to 630 nm and set zero with distilled water.
2. Operation table ( Add the following reagents to 1.5mL EP tube )

Reagent name (μL)	Blank tube (B)	Test tube (T)	Standard tube(S)
Extract solution	400	-	-
Sample	-	400	-
NH <sub>4</sub> <sup>+</sup> standard	-	-	400
Reagent I	400	400	400
Reagent II	400	400	400

Mix well and react in 37°C water bath for 20 min. Take 1 mL to the glass cuvette, and read the absorbance at 630 nm wavelength, which was recorded as A<sub>B</sub>, A<sub>T</sub> and A<sub>S</sub>. Calculate  $\Delta A_T = A_T - A_B$ ,  $\Delta A_S = A_S - A_B$ . The blank tube and standard curve only need to be measured 1-2 times.

## III. Calculate of Plant Ammonium Nitrogen Content

Plant Ammonium Nitrogen Content (μmol/g mass) =  $\Delta A_T \div \Delta A_S \times C_S \times V_E \div W \times F = 0.5 \times \Delta A_T \div \Delta A_S \div W \times F$

C<sub>S</sub>: Concentration of standard solution, 0.5μmol/L;

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

W: Sample mass, g;

F: Sample dilution multiple;

### Note:

1. The reagentI is used as soon as possible after preparation. If it is found to be discolored, it cannot be used.
2. If  $\Delta A > 1.0$ , it is recommended to reduce the sample size, increase the volume of the extract solution or dilute the supernatant for determination ; if  $\Delta A < 0.01$ , the sample quality extraction can be improved. Pay attention to the synchronous modification of the calculation formula, multiplied by the dilution factor.

### 1. Experimental example:

1. Take 0.1057g of *Albizia julibrissin Durazz.* root, add 1 mL of extract solution to the homogenate with ice bath,take the supernatant and follow the determination steps.The  $\Delta A_T = A_T - A_B = 0.245 - 0.009 = 0.236$ ,  $\Delta A_S = A_S - A_B = 0.648 - 0.009 = 0.639$ , measured by 1 mL glass cuvette, and ammonium nitrogen content is calculated according to the sample mass:

ammonium nitrogen content (μmol/g mass) =  $0.5 \times \Delta A_T \div \Delta A_S \div W \times F = 1.75$  μmol/g mass.

2. Take 0.1057g of *Prunus triloba Lindl.* leaves, add 1 mL of extract solution to the homogenate with ice bath, take the supernatant and dilute it with distilled water for 10 times and follow the

determination steps. The  $\Delta A_T = A_T - A_B = 0.791 - 0.009 = 0.782$ ,  $\Delta A_S = A_S - A_B = 0.648 - 0.009 = 0.639$ , measured by 1 mL glass cuvette, and ammonium nitrogen content is calculated according to the sample mass:

ammonium nitrogen content ( $\mu\text{mol/g mass}$ ) =  $0.5 \times \Delta A_T \div \Delta A_S \div W \times F = 60.88 \mu\text{mol/g mass}$ .

**Related Products:**

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|---------------|--|
| BC0080/BC0085 | Nitrate Reductase(NR) Activity Assay Kit                             |
| BC4960/BC4965 | Nitrate Reductase (NR)Activity Assay Kit(Griess-Colorimetric Method) |
| BC1500/BC1505 | Plant Nitrate Nitrogen Content Assay Kit                             |
| BC5480/BC5485 | Nitric Oxide(NO)Content Assay Kit                                    |
| BC1540/BC1545 | Nitrite Reductase (NiR) Activity Assay Kit                           |