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/Microplate reader

Cat No: BC6045

Size: 100T/96S

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 110 mL×1	2-8℃
Reagent IA	Liquid 2.5 mL×1	2-8℃
Reagent IB	Liquid 10 mL×1	2-8℃
Reagent II	Liquid 12mL×1	2-8℃
Standard	Liquid 1 mL×1	2-8℃

Solution Preparation:

1. Reagent I: Before use, according to the sample size, the ratio of reagent IA : reagent IB = 100μL : 400μL (0.5mL, about 5T) was prepared and used;
2. Standard: 100μmol/mL NH_4^+;
3. 0.5μmol/mL NH_4^+:Before use, add 100μL of 100μmol/mL NH_4^+ to 900μL of distilled water to get 10μmol/mL NH_4^+, then add 50μL of 10μmol/mL NH_4^+ to 950μL of distilled water to get 0.5μmol/mL NH_4^+, 0.5μmol/mL NH_4^+ 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/Microplate reader, water-bath/constant temperature incubator,, tabletop centrifuge, adjustable pipette, 96 well plate, 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/microplate reader for more than 30 minutes, adjust wavelength to 630 nm and set spectrophotometer 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	100	-	-
Sample	-	100	-
NH_4^+ standard	-	-	100
Reagent I	100	100	100
Reagent II	100	100	100
Mix well and react in 37°C water bath for 20 min. Take to the micro glass cuvette/96 well plate, and read the absorbance at 630 nm wavelength, which was recorded as A_B, A_T and A_S. 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_S: Concentration of standard solution, 0.5μmol/L;

V_E: 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 $\Delta A>0.7$, 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 0.1005g of *Crassulaceae J. St.-Hil.* root, add 1 mL of extract solution to the homogenate with ice bath,take the supernatant and follow the determination steps.The $\Delta A_T=A_T-A_B=0.791-0.054=0.737$, $\Delta A_S=A_S-A_B=0.470-0.054=0.416$, measured by 96 well plate, and ammonium nitrogen content is calculated according to the sample mass:
   ammonium nitrogen content (μmol/g mass) =$0.5\times\Delta AT\div\Delta AS\div W\times F=8.81$ μmol/g mass.
2. Take 0.1024g of *Prunus triloba Lindl.* leaves, add 1 mL of extract solution to the homogenate with ice bath, take the supernatantand dilute it with distilled water for 10 times and follow the

determination steps.The $\Delta A_T = A_T - A_B = 0.597 - 0.054 = 0.543$, $\Delta A_S = A_S - A_B = 0.470 - 0.054 = 0.416$, measured by 96 well plate, and ammonium nitrogen content is calculated according to the sample mass:

ammonium nitrogen content (μmol/g mass) $= 0.5 \times \Delta A_T \div \Delta A_S \div W \times F = 63.73$ μmol/g mass.

Related Products:

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