Plant Ammoniacal Nitrogen Assay Kit

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

Operation Equipment: Spectrophotometer/ microplate reader

Catalog Number: NA0505

Size:100T/96S

Components:

Extract solution: 120 mL×1 bottle, storage at 4℃.

Reagent I: powder×1 bottle, storage at 4℃, dissolve with Reagent III thoroughly before use, use up in 10 days.

Reagent II: powder×2 bottle, storage at 4℃ protect from light, dissolve with 1 ml thoroughly before use.

Reagent III: 22 mL×1 bottle, storage at 4℃.

Standard: powder×1 bottle, 10 mg cysteine, storage at 4℃ protect from light, Add 1.157 mL Extract solution to make 1000 μg/mL nitrogen standard solution.

Product Description:

Nitrogen is an essential element of living organisms. Ammonium nitrogen enters plant cells and forms amino acids or amides. Ammonia nitrogen content in plant tissues can reflect the degree of plant stress.

Alpha - amino acid can react with hydrated indene triketone to forms blue-purple compound which has characteristic at 570 nm. The amino acid content was calculated by measuring absorbance at 570 nm.

Reagents and Equipments Required but Not Provided:

Spectrophotometer/ microplate reader, micro glass cuvette/96 well flat-bottom plate, transferpettor, water bath, desk centrifuge, mortar/ homogenizer, anhydrous ethanol, ice and distilled water.

Procedure：

I. Sample preparation:

Add 1 mL Extract solution into 0.1 g tissue, homogenate at RT, 12000 g 25℃ centrifuge for 10 min and take the supernatant on ice for test.

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 570 nm, set the counter to zero with distilled water.
2. Dilute 1000 μg/mL nitrogen standard solution with Extract solution to 200 μg/mL, 100 μg/mL, 50 μg/mL, 25 μg/mL, 12.5 μg/mL for use.
3. Add the following reagents:

Reagent name (μL)	Test tube A_T	Standard tube A_S	Blank tube A_B
Sample	15	-	-
Standard solution	-	15	-

distilled water	-	-	15
Reagent I	150	150	150
Anhydrous ethanol	150	150	150
Reagent II	15	15	15

Mix thoroughly, cover cup with sealing film tightly, keep in boiling water for 10 min, reverse the EP tube some times after cooling, 8000 rpm centrifuge for 5 min, take 200 μL to ultra-micro glass cuvette/96 well flat-bottom plate, detect absorbance at 570 nm. Detect within 30 min, calculate ΔA(standard)= ΔA(S）=A_S-A_B, ΔA(test)= ΔA(T）= A_T-A_B.

III. Calculation:

1. Make standard curve:

Nitrogen standard liquid as the abscissa, ΔA(S) as ordinate, establish the standard curve, get formula y=kx +b, take ΔA(test) to formula, get x (μg/mL).

2. Calculation of NH_3-N content

A. Sample weight:

NH_3-N (μg/g FW)= x×Ve÷ W=x÷W

B. Protein concentration:

NH_3-N (μg/mg prot) = x×Ve÷ (Cpr×Ve)= x÷Cpr

Cpr: Protein concentration (mg/mL);

W: Sample weight (g);

Ve: Extract solution volume, 1 mL;

Note:

In order to ensure the accuracy of the experimental results, we need to take 1-2 samples for pre-experiment. If the absorbance is too high (higher than 0.6), dilute the extract and then determine.

Technical Specifications：

Minimum Detection Limit：24.2 μmol/mL

Linear Range：25-400 μmol/mL

Recent Product citations：

[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：

NA0865/NA0622 Nitrate Reductase(NR) Activity Assay Kit

NA0754/NA0512 Glutaminase (GLS) Assay Kit

NA0753/NA0511 Glutamate dehydrogenase (GDH) Activity Assay Kit