（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K859-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader (615-635 nm)

Detection range：0.046-1 mmol/L

Elabscience® Plant Ammonium Nitrogen Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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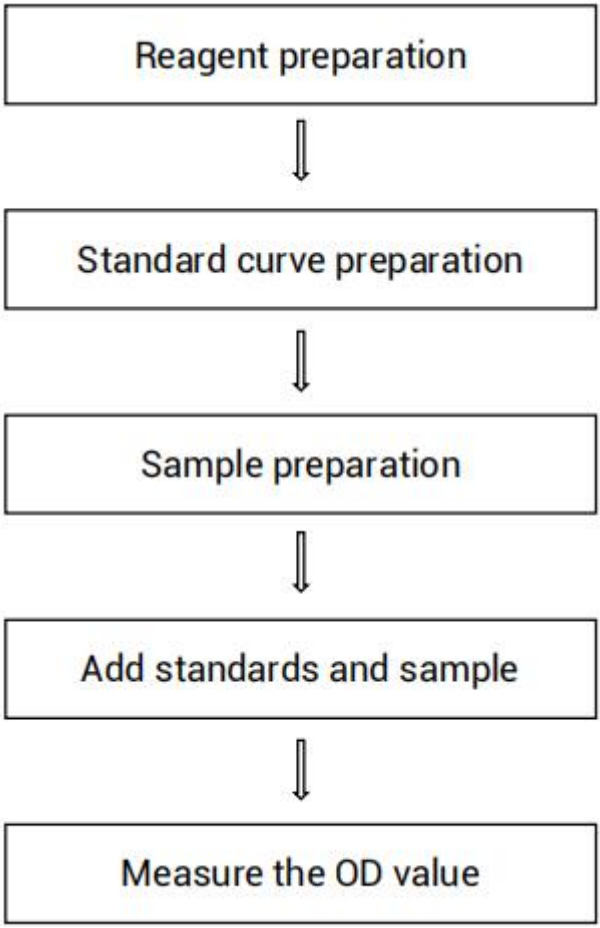
Assay summary



Intended use

This kit can be used to detect the ammonium nitrogen content in plant tissue samples.

Detection principle

Ammonium nitrogen reacts with hypochlorite and phenolic substances in a strong alkaline medium to produce water-soluble dye indigophenol blue, which has a characteristic absorption peak at 625 nm, and the absorption value is proportional to the content of ammonium nitrogen.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	55 mL × 1 vial	55 mL × 2 vials	2-8°C, 12 months, shading light
Reagent 2	Chromogenic Agent A	7.5 mL × 1 vial	15 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	Chromogenic Agent B	7.5 mL × 1 vial	15 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	5 mmol/L Standard	1 mL × 1 vial	1 mL × 1 vial	2-8°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (615-635 nm, optimum wavelength: 625 nm)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of 1 mmol/L standard solution：

For each well, prepare 2000 μL of 1 mmol/L standard solution (mix well

1600 μL of extracting solution and 400 μL of 5 mmol/L standard). The 1 mmol/L standard solution should be prepared on spot.

③ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 mmol/L standard solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.10, 0.20, 0.30, 0.40, 0.60, 0.80, 1.00 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0.00**	**0.10**	**0.20**	**0.30**	**0.40**	**0.60**	**0.80**	**1.00**
1 mmol/L Standard (μL)	0	50	100	150	200	300	400	500
Extracting solution (μL)	500	450	400	350	300	200	100	0

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL extracting solution with a dounce homogenizer at 4℃.

③ Centrifuge at 12000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Determine the protein concentration of supernatant (E-BC-K168-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Epipremnum aureu root tissue homogenization	1
10% Pumpkin tissue homogenization	1
10% Corn leaf tissue homogenization	1
10% Green peppers tissue homogenization	1

Note: The diluent is extracting solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

Laboratory equipment must be clean to avoid introducing contamination.

Operating steps

① Standard well: Add 20 μL of standard solution with different concentrations to the corresponding wells.

Sample well: Add 20 μL of sample to the corresponding wells.

② Add 120 μL of chromogenic agent A to each well.

③ Add 120 μL of chromogenic agent B to each well.

④ Mix fully with microplate reader for 10 s and incubate at 25°C for 20 min. Measure the OD value of each well at 625 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Tissue sample (The tissue wet weight was calculated):

ammonium nitrogen content（μg/g） = (ΔA - b) ÷ a × V ÷ m × f × 14*

2. Tissue sample (The tissue protein was calculated):

ammonium nitrogen content（μg/mgprot） = (ΔA - b) ÷ a ÷ C_{pr} × f × 14*

[Note]

ΔA: OD_{sample} – OD_{blank}.

C_{pr}: The concentration of protein in sample, mgprot/mL.

f: Dilution factor of sample before test.

V: The volume of extracting solution in the preparation step of sample, mL.

m: Tissue wet weight, g.

14: The micromolar mass of nitrogen, 14 μg/μmol.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three pumpkin tissue samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.20	0.40	0.80
%CV	2.2	1.4	3.9

Inter-assay Precision

Three pumpkin tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.20	0.40	0.80
%CV	4.9	1.6	3.5

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 96%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	0.20	0.40	0.80
Observed Conc. (U/L)	0.19	0.39	0.74
Recovery rate (%)	94	97	98

Sensitivity

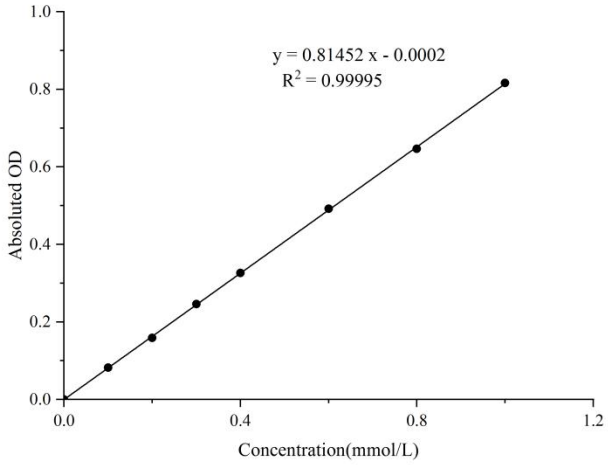
The analytical sensitivity of the assay is 0.046 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the

corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (mmol/L)	**0.00**	**0.10**	**0.20**	**0.30**	**0.40**	**0.60**	**0.80**	**1.00**
OD value	0.044	0.128	0.214	0.290	0.379	0.549	0.703	0.858
	0.043	0.126	0.210	0.286	0.371	0.530	0.689	0.836
Average OD	0.044	0.127	0.212	0.288	0.375	0.540	0.696	0.847
Absoluted OD	0.000	0.084	0.169	0.245	0.332	0.496	0.653	0.804



Appendix Π Example Analysis

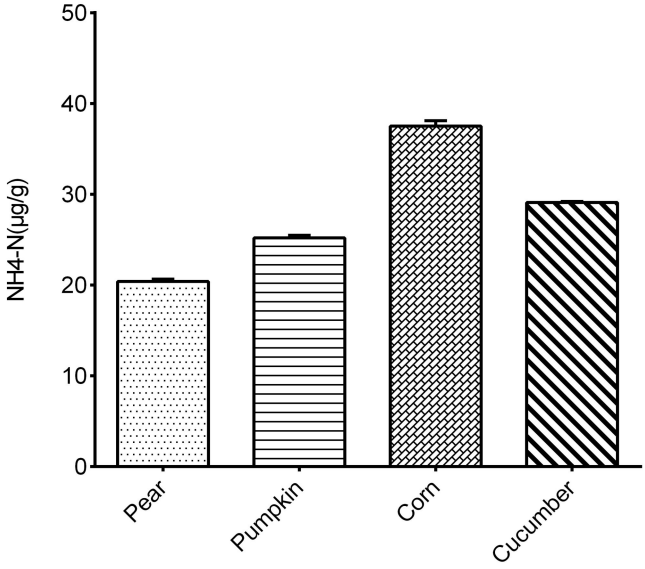
Example analysis：

Take 20 μL of 10% green peppers tissue homogenization and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.81452 x - 0.0002. The OD value of sample is 0.156, the OD value of blank is 0.050, and the calculation result is:

ammonium nitrogen content（μg/g）= (0.156 - 0.044 + 0.0002) ÷ 0.81452 × 0.9 ÷ 0.1×14 = 17.36 μg

Detect 10% pear tissue homogenization, 10% pumpkin tissue homogenization, 10% corn tissue homogenization and 10% cucumber tissue homogenization, according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.