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

Catalog No：E-BC-K858-M

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

Measuring instrument：Microplate reader (405-415 nm)

Detection range：1.09-200 μg/mL

Elabscience® Plant Nitrate 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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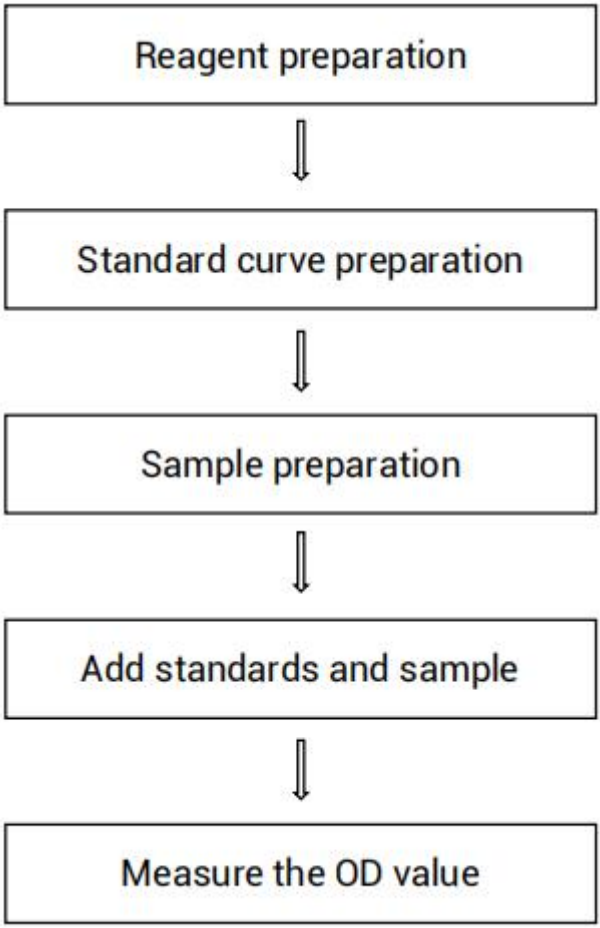
Assay summary



Intended use

This kit can be used to measure nitrate nitrogen content in plant tissue and soil samples.

Detection principle

Nitrate nitrogen can react with salicylic acid to produce nitrosalicylic acid in a strongly acidic environment. Nitrosalicylic acid is yellow under strong alkaline conditions, and the depth of color is proportional to the content at a wavelength of 410 nm.

Kit components & storage

Item	Component	Size 1 (48T)	Size 2 (96T)	Storage
Reagent 1	Chromogenic Agent 1	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 months, shading light
Reagent 2	Chromogenic Agent 2	60 mL × 1 vial	60 mL × 2 vials	2-8℃, 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 (405-415 nm, optimum wavelength: 410 nm), Incubator, Vortex mixer, Centrifuge

Reagents:

Potassium nitrate, Concentrated sulfuric acid

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of reaction solution：

Dissolve one vial of chromogenic agent 1 with 2.4 mL of concentrated sulfuric acid, mix well to dissolve and stand until the solution looks clear. Store at 4°C for a week protected from light.

③ The preparation of 500 μg/mL standard solution：

Dissolve 3.61 mg of potassium nitrate with 1 mL of double distilled water, mix well to dissolve. Store at -20°C for a week protected from light.

④ The preparation of standard curve：

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

Dilute 500 μg/mL standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 80, 120, 160, 200 μg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μg/mL)	**0**	**10**	**20**	**40**	**80**	**120**	**160**	**200**
500 μg/mL standard (μL)	0	10	20	40	80	120	160	200
Double distilled water (μL)	500	490	480	460	420	380	340	300

Sample preparation

① Sample preparation

Tissue sample:

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

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 100 mg tissue in 400 μL double distilled water with a dounce homogenizer at 4℃.

④ The homogenate was then placed in a boiling water bath to extract for 30 min, and then the sample was rinsed in running water to cool to 25℃.

⑤ Centrifuge at 10000×g for 15 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

Soil samples:

① Fresh soil samples were air-dried naturally or oven-dried at 37℃ and sieved through 30 to 50 mesh.

② Homogenize 100 mg soil in 400 μL double distilled water with a dounce homogenizer at 4℃.

③ Centrifuge at 10000×g for 15 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

② Dilution of sample

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

Sample type	Dilution factor
20% Epipremnum aureum root tissue homogenization	1
20% Soil homogenization	1
20% Core leaves tissue homogenization	1
20% Green pepper tissue homogenization	1

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

The key points of the assay

① The reaction solution should be mixed fully.
② The viscosity of reaction solution is very high, so should be pipetted slowly and carefully.
③ The reaction solution and chromogenic agent 2 are strong corrosive liquid, please pay attention to protection during operation.

Operating steps

① Standard tube: Add 10 μL of standard with different concentrations to the corresponding tubes;
Sample tube: Add 10 μL of sample to the corresponding tubes.
② Add 50 μL of reaction solution to each tube.
③ Mix fully and stand for 3 min at room temperature.
④ Add 950 μL of chromogenic agent 2 to each tube.
⑤ Mix fully and take 200 μL the solution of each tube to the microplate with a micropipette, measure the OD value of each well at 410 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:

Tissue and soil samples:

$$\text{nitrate nitrogen content (μg/g)} = (\Delta A - b) \div a \times V \div m \times f$$

[Note]

ΔA: Absolute OD, $OD_{Sample} - OD_{Blank}$.

f: Dilution factor of sample before test.

m: The wet weight of tissue or soil, g.

V: The volume of double distilled water in sample preparation, mL.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three core leaves 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 (μg/mL)	52.50	98.40	120.50
%CV	1.5	3.2	4.3

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μg/mL)	52.50	98.40	120.50
%CV	3.2	5.1	3.7

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%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μg/mL)	15	50	150
Observed Conc. (μg/mL)	14.1	48.0	147.0
Recovery rate (%)	94	96	98

Sensitivity

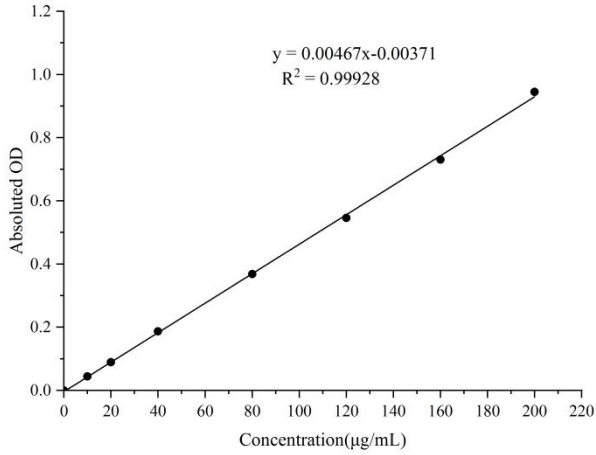
The analytical sensitivity of the assay is 1.09 μg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding

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 (μg/mL)	0	10	20	40	80	120	160	200
OD value	0.045	0.089	0.135	0.229	0.420	0.599	0.775	0.994
	0.047	0.091	0.135	0.236	0.408	0.584	0.779	0.988
Average OD	0.046	0.090	0.135	0.233	0.414	0.592	0.777	0.991
Absoluted OD	0.000	0.044	0.089	0.187	0.368	0.546	0.731	0.945



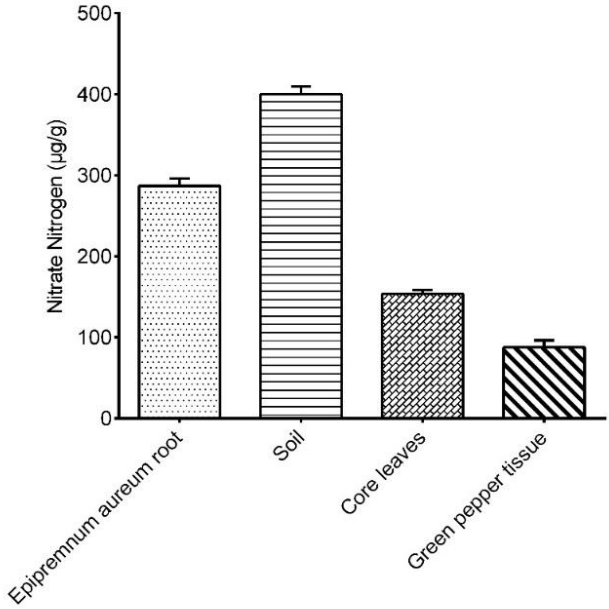
Appendix Π Example Analysis

Example analysis：

Take 10 μL of 20% epipremnum aureum root tissue homogenization, carry the assay according to the operation steps. The results are as follows: standard curve: y = 0.0047 x - 0.0037, the average OD value of the sample is 0.310, the average OD value of the blank is 0.046, and the calculation result is:

nitrate nitrogen content (μg/g) = (0.310 - 0.046 + 0.0037) ÷ 0.0047 × 0.4 ÷ 0.1 = 227.83 μg/g

Detect 20% epipremnum aureum root tissue homogenization, 20% soil homogenization, 20% core leaves tissue homogenization and 20% green pepper 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.