CheKine™ Micro Plant Ammonium Nitrogen Assay Kit

Cat #: KTB3081 Size: 48 T/48 S 96 T/96 S

	Micro Plant Ammonium Nitrogen Assay Kit		
REF	**Cat #:** KTB3081	LOT	**Lot #:** Refer to product label
	Detection range: 0.039-2.5 μmol/mL		**Sensitivity:** 0.039 μmol/mL
	Applicable sample: Plant Tissues		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Ammonium nitrogen can be directly absorbed and utilized by plants, or converted into nitrate nitrogen by nitrifying microorganisms, and then assimilated into organic nitrogen compounds by plants or microorganisms. Ammonium nitrogen can react with sodium hypochlorite and phenol in strong alkaline medium to produce water-soluble blue dye indophenol blue. The product has a characteristic absorption peak at 625 nm, and the absorbance value is proportional to the content of ammonium nitrogen.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	70 mL	70 mL×2	4℃
Reagent Ⅰ	Powder×2 vials	Powder×2 vials	4℃, protected from light
Reagent Ⅱ	6 mL	12 mL	4℃, protected from light
Reagent Ⅲ	1.5 mL	3 mL	4℃
Standard (500 μmol/mL)	1 mL	1 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 625 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Thermostatic shaker, centrifuge
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Reagent I: Prepare before use, according to the experimental requirements, take one vial of Reagent I and dissolve it completely in 6 mL of deionized water. Prepare and use immediately.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Standard (500 μmol/mL): Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Standard preparation: Use 500 μmol/mL standard, prepare standard curve dilution as described in the table.

Num.	Standard Volume	Extraction Buffer Volume (μL)	Concentration (μmol/mL)
Std.1	5 μL 500 μmol/mL	995	2.5
Std.2	100 μL of Std.1 (2.5 μmol/mL)	100	1.25
Std.3	100 μL of Std.2 (1.25 μmol/mL)	100	0.625
Std.4	100 μL of Std.3 (0.625 μmol/mL)	100	0.313
Std.5	100 μL of Std.4 (0.313 μmol/mL)	100	0.156
Std.6	100 μL of Std.5 (0.156 μmol/mL)	100	0.078
Std.7	100 μL of Std.6 (0.078 μmol/mL)	100	0.039
Blank	0	100	0

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.

Add extraction buffer according to the ratio of plant sample mass (g): Extraction Buffer volume (mL) of 1:5-10 (It is recommended to weigh about 0.1 g plant sample and add 1 mL of Extraction Buffer volume). Homogenize at room temperature, centrifuge at 10,000 g for 10 min at 4℃, and keep the supernatant for test.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 625 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (The following were operated in the 96-well plate or microglass cuvette):

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Supernatant	0	0	20
Extraction Buffer	20	0	0
Standard	0	20	0
Reagent I	80	80	80
Reagent II	80	80	80
Mix thoroughly and keep at 25℃ for 1 h			

Reagent III	20	20	20

3. After thorough mixing, measure the absorbance value at 625 nm immediately, record it as A_{Blank}, $A_{Standard}$ and A_{Test}. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Blank well and standard well only need to measure 1 time. In order to guarantee the accuracy of experimental results, pre-experiment for 2-3 samples with potential significant difference was recommended. If ΔA_{Test} is less than 0.04, increase the sample amount appropriately. If ΔA_{Test} is greater than 2.0, dilute the sample further with Extraction Buffer, and multiply the calculated result by the dilution factor, or reduce the amount of sample used for extraction.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve:

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve, get the standard equation, and bring the ΔA_{Test} into the equation to get the x value (μmol/mL).

2. Calculation of ammonium nitrogen content:

Ammonium nitrogen content (μg/g fresh weight)=$x \times V_{Total\ sample} \times 18 \div W$**$=180 \times x$**

$V_{Total\ sample}$: added Extraction Buffer volume, 1 mL; W: sample weight, 0.1 g; 18: molar mass of NH_4^+, μg/μmol.

Typical Data

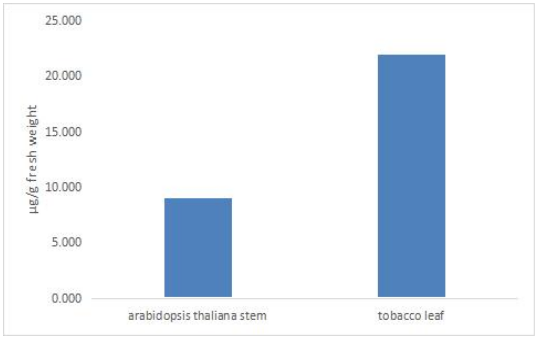

Figure 1. Determination ammonium nitrogen in plant samples by this assay kit

Recommended Products

Catalog No.	Product Name
KTB3080	CheKine™ Micro Plant Nitrate Nitrogen Assay Kit
KTB1540	CheKine™ Micro Plant Total Phenols (TP) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.