CheKine™ Micro Plant Nitrate Nitrogen Assay Kit

Cat #: KTB3080 Size: 96 T/96 S

	Micro Plant Nitrate Nitrogen Assay Kit		
REF	**Cat #:** KTB3080	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant Tissue		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Nitrate nitrogen (NO_3^--N) is the main nitrogen source for plants, and the nitrate nitrogen content in plants reflects the supply of nitrate nitrogen in the soil, which can be used as an indicator of soil nitrogen fertilizer. Under concentrated acid conditions, NO_3^--N reacts with salicylic acid to produce nitrosalicylic acid. Under alkaline conditions (pH>12), nitrosalicylic acid turns yellow, and absorbance changes reflect the concentration of NO_3^--N within a certain range, Thus the nitrate nitrogen content can be calculated by colorimetric measurement.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
Reagent Ⅰ	Powder×2 vials	4℃, protected from light
Reagent Ⅱ	60 mL	4℃
Standard	Powder×1 vial	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 410 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Analytical balance, thermostatic water bath, thermostatic shaker, centrifuge
- Deionized water, sulfuric acid, activated charcoal
- Homogenizer (for tissue samples)

Reagent Preparation

Working Reagent Ⅰ: Prepare before use, according to the experimental requirements, take one vial of Reagent I and dissolve it

completely in 1.2 mL of sulfuric acid. Use the prepared solution as soon as possible. It can be stored at 4℃, protected from light, for up to 1 week.

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

Note: Both Working Reagent Ⅰ and Reagent Ⅱ are highly corrosive. Take appropriate protective measures when handling them.

Standard: Before use, prepare 1 mL of deionized water to prepare 1 mg/mL of NO_3^--N standard solution. Store at 4℃ for 1 month.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for 1 month.

According to the ratio (1:5-10) of plant tissue (g): deionized water (mL) (Generally, it is recommended to weigh about 0.1 g and add 1 mL of deionized water). Add deionized water, homogenize at room temperature, and then keep the homogenate in a 90℃ constant temperature water bath for 30 min and shake regularly or in a 90℃ constant temperature shaking incubator for 30 min. After cooling, centrifuge at 25℃ for 15 min at 12,000 g, and take the supernatant for test. (Add 3 mg activated charcoal into the homogenate for dark plant before extraction).

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 410 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (the following operations are performed in a 1.5 mL centrifuge tube):

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)
Supernatant	0	0	10
Deionized Water	10	0	0
Standard	0	10	0
Reagent Ⅰ	20	20	20
Mix thoroughly and keep 30 min at 25℃			
Reagent Ⅱ	475	475	475

3. Mix well, vortex to fully dissolve the precipitate, take 200 μL to microglass cuvette/96 well plate, and measure the absorbance at 410 nm. The absorbance of blank well, standard well, test well recorded as A_{Blank}, $A_{Standard}$ and A_{Test}. Finally, 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 A_{Test} is greater than 1.5, it is recommended to dilute the sample with deionized water before measurement.

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.

NO_3^--N content (μg/g sample) $=\Delta A_{Test} \div (\Delta A_{Standard} \div C_{Standard}) \times V_{Total} \div W=$**$1{,}000 \times \Delta A_{Test} \div \Delta A_{Standard} \div W$**

W: sample mass, g; $C_{Standard}$: concentration of standard solution, 1,000 μg/mL; V_{Total}: volume of extraction solution, 1 mL.

Typical Data

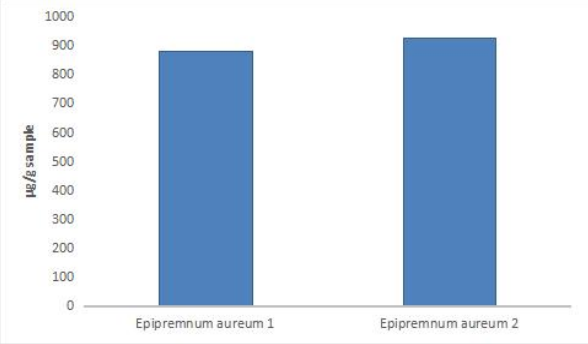

Figure 1. Determination of nitrate nitrogen content in *Epipremnum Aureum* leaves

Recommended Products

Catalog No.	Product Name
KTB1320	CheKine™ Micro Plant Soluble Sugar Assay Kit
KTB1520	CheKine™ Micro Plant Oligomeric Proantho Cyanidins (OPC) 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.