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## CheKine™ Micro Plant Nitrate Nitrogen Assay Kit

Cat #: KTB3080 Size: 96 T

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

## **Assay Principle**

Nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-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<sub>3</sub><sup>-</sup>-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<sub>3</sub><sup>-</sup>-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	1×2	4°C, protected from light	
Reagent	50 mL	4°C	
Standard	1	4°C	

## **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

#### **Reagent Preparation**

**Reagent I:** Prepare before use, add 1 mL of sulfuric acid to each tube according to the dosage and fully dissolve before use, ready to use; Store at 4°C away from light.

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



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## **Sample Preparation**

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°C constant temperature water bath for 30 min and shake regularly or in a 90°C constant temperature shaking incubator for 30 min. After cooling, centrifuge at 25°C 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 Well (µL)	Standard Well (µL)	Test Well (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°C					
Reagent II	475	475	475		

<sup>3.</sup> Mix well, vortex to fully dissolve the precipitate, take 200  $\mu$ L 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.

#### **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) = $\Delta A_{Test}$ + ( $\Delta A_{Standard}$ + $C_{Standard}$ ) × $V_{Total}$ +W=1,000× $\Delta A_{Test}$ + $\Delta A_{Standard}$ +W

W: sample mass, g; C<sub>Standard</sub>: concentration of standard solution, 1,000 μg/mL; V<sub>Total</sub>: volume of extraction solution, 1 mL.



# **Typical Data**

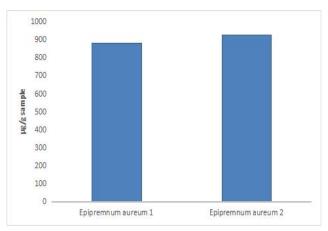


Figure 1. Determination of nitrate nitrogen content in *Epipremnum Aureum* leaves

## **Precautions**

- 1. Prepare Reagent | before use, and should be used as soon as possible.
- 2. Reagent | and Reagent || possess corrosion properties, proper protective measures were needed.
- 3. Activated charcoal was required for dark plant.

#### **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.

