EZMeta™ Plant Nitrate Nitrogen Colorimetric Assay Kit

Cat #: D-AKC3080

Size: 96T

Storage: Stored at 4°C for 12 months, protected from light

Product Information

Applicable samples: Plant Tissue

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	1×2	4°C, protected from light
Reagent Ⅱ	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.

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

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

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.

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) $=\Delta A_{Test} \div (\Delta A_{Standard} \div C_{Standard}) \times V_{Total} \div W=\mathbf{1{,}000 \times \Delta A_{Test} \div \Delta A_{Standard} \div W}$

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

Typical Data

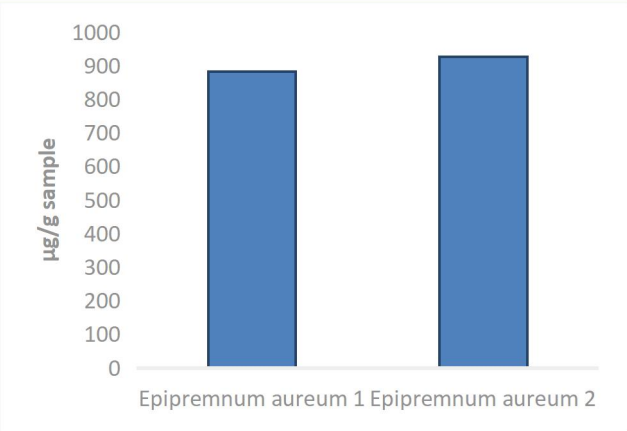

Figure 1. Determination of nitrate nitrogen content in *Epipremnum Aureum* leaves

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.