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Plant Nitrate Nitrogen Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: AK0427

Size:100T/48S

Components:

Reagent I: powder×2 bottle, storage at 4°C protected from light; Add 0.5 mL concentrated sulfuric acid to each bottle according to dosage before use.

Reagent II: liquid 50 mL×1 bottle, storage at 4°C

Standard: powder×1 bottle, storage at 4°C, 10 mg KNO₃. Dissolve thoroughly with 0.935 mL distilled water before use, to make 1400 μg/mL NO₃-N standard solution.

Product Description:

Nitrate is one of the nitrogen - containing substances absorbed by plants. Nitrate is reduced in roots, branches or leaves, depending on plant type and environmental conditions. Detecting nitrate nitrogen content in plants is significant to understand the nitrogen metabolism mechanism.

NO₃⁻ can react with salicylic acid to form nitrosalicylic acid under the condition of concentrated acid, which shows yellow under the condition of pH>12. Within a certain range, the color depth is proportional to the content.

Reagents and Equipments Required but Not Provided:

Spectrophotometer/microplate reader, micro glass cuvette/96 well flat-bottom plate, water bath, desk centrifuge, mortar/homogenizer, ice and distilled water.

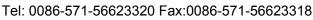
Sample preparation:

Add 1 mL distilled water into 0.1 g tissue, fully grinding at RT and put it in 90°C water bath for 30 min, shaking during the time. Or put in 90°C shaker, centrifuge at 12000 g, 25°C for 15 min after cooling. Take the supernatant on ice for test.

Procedure:

- 1. Preheat spectrophotometer/ microplate reafer for 30 min, adjust the wavelength to 410 nm, set the counter to zero with distilled water.
- 2. Dilute 1400 μg/mL NO₃-N standard solution with distilled water to 28 μg/mL for use.
- 3. Add the following reagents:

П					
	Reagent (µL)	Blank tube A2	Standard tube A1	Test tube A3	Control tube A4





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Sample			8	8		
Standard		8				
Distilled water	8			12		
Reagent I	12	12	12	_		
Mix thoroughly, stand at 37°C for 30 min.						
Reagent II	280	280	280	280		

Mix thoroughly, shaking until the sediment dissolve thoroughly, take 200 µL to micro glass cuvette/96 well flat-bottom plate, detect absorbance at 410 nm, $\Delta A(\text{standard}) = \Delta A(S) = A1 - A2$, $\Delta A(\text{test}) = \Delta A(T) = A3 - A4$.

Calculation:

1. Sample weight:

NO₃-N (
$$\mu$$
g/g weight) = Δ A(T)÷(Δ A(S) ÷C) ×Ve÷ W =28× Δ A(T) ÷ Δ A(S) ÷W

2. Protein concentration:

$$NO_3-N \text{ (} \mu\text{g/mg prot)} = \Delta A(T) \div (\Delta A(S) \div C) \times Ve \div (Cpr \times Ve) = 28 \times \Delta A(T) \div \Delta A(S) \div Cpr$$

C: Standard concentration, 28 µg/mL;

Cpr: Sample concentration (mg/mL);

W: Sample weight (g);

Ve: Extraction volume, 0.3 mL;

Note:

- 1. Use Reagent I as soon as possible, storage at 4°C for one week;
- 2. Both Reagent I and Reagent II are highly corrosive, and protective measures must be taken during operation.
- 3. If $\Delta A(T) > 1$, dilute the sample before the determination.

Technical Specifications:

Minimum Detection Limit: 0.4631 ug/mL

Linear Range: 3.5-140 ug/mL

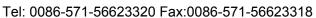
References:

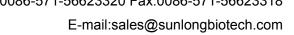
[1] Fuyuan Zhu, Moxian Chen, Wailung Chan, et al. SWATH-MS quantitative proteomic investigation of nitrogen starvation in Arabidopsis reveals new aspects of plant nitrogen stress responses. Journal of Proteomics. September 2018;(IF3.537)

Experimental example:

1. Take 0. 1g apple to 1ml distilled water, operate as the procedure after taking the supernatant, test and calculate \triangle A(test)= \triangle A(T)=A3-A4=0.333-0.051=0.282, \triangle A(standard)= \triangle A(S)=A1-A2=0.320-0.048=0.272, calculate content by sample weight: NO3-N (μ g/g weight)= 28* Δ A \div Δ A(S) \div W =28 \times

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 $0.282 \div 0.272 \div 0.1 = 290.3 \mu \text{ g/g weight.}$

2. Take 0. 1g leaf to 1 ml distilled water, operate as the procedure after taking the supernatant, test and calculate \triangle A(test)= \triangle A(T)=A3-A4=0.633-0.458=0.175, \triangle A(standard)= \triangle A(S)=A1-A2=0.320-0.048=0.272, calculate content by sample weight: NO3-N (μ g/g weight)= 28* \triangle A÷ \triangle A(S)÷ W=28× 0.175÷0.272÷0.1=180.1 μ g/g weight.

Related products:

AK0301/AK0300 Nitrate Reductase(NR) Activity Assay Kit

AK0436/AK0435 Glutaminase(GLS) Activity Assay Kit

AK0434/AK0433 Glutamic Acid Dehydrogenase(GDH) Activity Assay Kit