Plant Nitrate Nitrogen Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: NA0749

Size:50T/24S

Components:

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

Reagent II: liquid 100 mL×1 bottle, storage at 4℃.

Standard: powder×1 bottle, storage at 4℃, 10 mg KNO_3. Dissolve thoroughly with 0.935 mL distilled water before use to make 1400 μg/mL NO_3-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.

NO_3^- 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, water bath, centrifuge, transferpettor, 1 mL glass cuvette, mortar/homogenizer, ice and distilled water.

Sample preparation:

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

Procedure:

1. Preheat spectrophotometer for 30 min, adjust the wavelength to 410 nm, set the counter to zero with distilled water.

2. Dilute 1400 μg/mL NO_3-N standard solution with distilled water to 28 μg/mL for use.

3. Add the following reagents:

Reagent (μL)	Blank tube A2	Standard tube A1	Test tube A3	Controlt tube A4
Sample			40	40
Standard		40		
Distilled water	40			60
Reagent I	60	60	60	
Mix thoroughly, stand at 25℃ for 30 min.				
Reagent II	1400	1400	1400	1400
Mix thoroughly, shaking until the sediment dissolved thoroughly, take 1 mL from 1 mL glass cuvette, detect absorbance at 410 nm, ΔA(standard)=ΔA(S)=A1-A2, ΔA(test)=ΔA(T) =A3-A4.				

Calculation:

1. Sample weight:

NO_3-N (μg/g weight)= ΔA(T)÷(ΔA(S) ÷C)×Ve÷ W =28×ΔA(T) ÷ΔA(S) ÷W

2. Protein concentration:

NO_3-N (μg/mg prot)= ΔA(T) ÷(ΔA(S) ÷C)×Ve÷ (Cpr×Ve)=28×ΔA(T) ÷ΔA(S) ÷Cpr

C: Standard concentration, 28 μg/mL

Cpr: Sample concentration (mg/mL);

W: Sample weight (g);

Ve: Extraction volume, 1 mL;

Note:

1. Use Reagent I as soon as possible, storage at 4℃ for one week;

2. Both Reagent I and Reagent II are highly corrosive, and protective measures must be taken during operation.

3. If ΔA(T)>1, dilute the sample before the determination.

Technical Specifications：

Minimum Detection Limit：0.7534 ug/mL

Linear Range：0.875-84 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)

Related products：

NA0865/NA0622 Nitrate Reductase(NR) Activity Assay Kit

NA0754/NA0512 Glutaminase(GLS) Activity Assay Kit

NA0753/NA0511 Glutamic Acid Dehydrogenase(GDH) Activity Assay Kit

Experimental example:

1. Take 0.1g apple to 1ml distilled water, operate as the procedure after taking the supernatant, test and calculate ΔA(test)=ΔA(T)=A3-A4=0.560-0.002=0.558, ΔA(standard)=ΔA(S)=A1-A2=0.563-0.01=0.553, calculate content by sample weight: NO3-N (μg/g weight)= 28*ΔA÷ΔA(S)÷W =28×0.558÷0.553÷0.1=282.5 μg/g weight.
2. Take 0.1g leaf to 1ml distilled water, operate as the procedure after taking the supernatant, test and calculate ΔA(test)=ΔA(T)=A3-A4=0.907-0.645=0.262, ΔA(standard)=ΔA(S)=A1-A2=0.563-0.01=0.553, calculate content by sample weight: NO3-N (μg/g weight)= 28*ΔA÷ΔA(S)÷W =28×0.262÷0.553÷0.1=132.7 μg/g weight.