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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℃ 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℃

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/ 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℃ water bath for 30 min, shaking during the time. 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/ microplate reafer 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	Control tube A4

Sample			8	8
Standard		8		
Distilled water	8			12
Reagent I	12	12	12	-
Mix thoroughly, stand at 37℃ 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, 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, 0.3 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：0.4631 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 Δ A(test)= Δ A(T)=A3-A4=0.333-0.051=0.282, Δ A(standard)= Δ A(S)=A1-A2=0.320-0.048=0.272, calculate content by sample weight: NO3-N (μ g/g weight)= 28* Δ A÷ Δ A(S)÷ W =28×

0.282÷0.272÷0. 1=290.3 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=0.633-0.458=0. 175, ΔA(standard)=ΔA(S)=A1-A2=0.320-0.048=0.272, calculate content by sample weight: NO3-N (μg/g weight)= 28* ΔA÷ ΔA(S)÷ W =28×0.175÷0.272÷0. 1=180. 1 μg/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