

Plant Nitrate Nitrogen Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: NA0507

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 reader 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:

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:

$$\text{NO}_3\text{-N } (\mu\text{g/g weight}) = \Delta A(T) \div (\Delta A(S) \div C) \times V_e \div W = 28 \times \Delta A(T) \div \Delta A(S) \div W$$

2. Protein concentration:

$$\text{NO}_3\text{-N } (\mu\text{g/mg prot}) = \Delta A(T) \div (\Delta A(S) \div C) \times V_e \div (C_{pr} \times V_e) = 28 \times \Delta A(T) \div \Delta A(S) \div C_{pr}$$

C: Standard concentration, 28 µg/mL;

C_{pr}: Sample concentration (mg/mL);

W: Sample weight (g);

V_e: 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 µg/mL

Linear Range: 3.5-140 µg/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 $\Delta A(\text{test}) = \Delta A(T) = A3 - A4 = 0.333 - 0.051 = 0.282$, $\Delta A(\text{standard}) = \Delta A(S) = A1 - A2 = 0.320 - 0.048 = 0.272$, calculate content by sample weight: $\text{NO}_3\text{-N } (\mu\text{g/g weight}) = 28 \times \Delta A \div \Delta A(S) \div W = 28 \times$

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

2. Take 0.1g leaf to 1ml distilled water, operate as the procedure after taking the supernatant, test and calculate $\Delta A(\text{test}) = \Delta A(\text{T}) = A_3 - A_4 = 0.633 - 0.458 = 0.175$, $\Delta A(\text{standard}) = \Delta A(\text{S}) = A_1 - A_2 = 0.320 - 0.048 = 0.272$, calculate content by sample weight: $\text{NO}_3\text{-N} (\mu \text{g/g weight}) = 28 * \Delta A \div \Delta A(\text{S}) \div W = 28 \times 0.175 \div 0.272 \div 0.1 = 180.1 \mu \text{g/g weight}$.

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