Plant Root Vitality Assay Kit (naphthylamine method)

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

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: NA0150

Size:100T/96S

Components:

Reagent I: 10 mL×1. Storage at 2-8℃.

Reagent II: 110 mL×2. Storage at 2-8℃.

Reagent III: 1 mL×1. Storage at 2-8℃. Before use,add 9mL of distilled water to reagent III, mix thoroughly. Unused reagent can be stored at 2-8℃ for 4 weeks.

Reagent IV: 10 mL×1. Storage at 2-8℃.

Standard: 2 mL×1. Storage at 2-8℃. 0.4 mg/mL α-naphthylamine standard solution.

Product Description:

The root system is the main organ for water and mineral nutrient absorption in plants, as well as the organ for synthesis, assimilation and transformation of important substances such as amino acids and hormones in the plant body. Therefore, the growth and activity of roots directly affect the growth, nutrient level and yield level of individual plants, and it is of practical importance to determine the root vitality.

Plant roots can oxidize α-naphthylamine adsorbed on the root surface to produce red α-hydroxy-1-naphthylamine, which is precipitated on the surface of oxidized roots, causing this part to be dyed red. α-Naphthylamine interacts with sodium p-aminobenzene sulfonate and nitrite under acidic conditions to produce a red azo dye. Root vitality can be expressed by the reduction of α-naphthylamine.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, water bath/incubator, adjustable pipette, filter paper, micro glass cuvette/96 well plate, distilled water..

Procedure

I. Sample processing :

Wash the root tissues, remove the soil from the roots, and gently wipe dry without overly squeezing to damage the root cells. Weigh 0.2 g in a 5 mL EP tube.

II. Determination procedure

a. Preheat the spectrophotometer /microplate reader 30 minutes, adjust wavelength to 520 nm, set zero with distilled water.

b. Standard solution

The 0.4 mg/mL α-naphthylamine standard solution was diluted with **reagent I** to 0.4、0.2、0.1、0.05、0.025、0.0125、0.00625、0.003125、0.0015625mg/mL of standard solution (0 mg/mL was recorded as standard blank tube).

c. Then operate according to the following table.(5mL EP tube)

Reagent name（μL）	Test tube(T)	Control tube(C)	Standard tube(S)
sample	0.2g	-	-
Reagent II	2000	2000	-
	The reaction was carried out at 37°C and protected from light. 75 μL of each reaction solution had to be aspirated at 10 min and 3h 10 min, respectively. Add Reagent III and Reagent IV to 2 new 1.5mL EP tubes according to the table below, and then aspirate 400μL of each reaction solution into the EP tubes. **Note:** Add Reagent III and Reagent IV first, then add this reaction solution, make sure not to reverse the order!		
Reagent III	75	75	75
Reagent IV	75	75	75
The above reaction solution	75	75	-
standard solution	-	-	75
Mix thoroughly, react at room temperature for 20 min, take 200μL in a micro glass cuvette/96 well plate, and measure the absorbance at 520 nm. the absorbance value corresponding to 10 min is recorded as A1, and the absorbance value corresponding to 3h10 min is recorded as A2. calculate ΔAt = A1t - A2t, ΔAc = A1c - A2c, Δ A = ΔAt- ΔAc, ΔA standard = A standard - A standard blank tube. (Standard curve and blank tube should be done only 1-2 times)			

III. Calculation formula

1. Drawing of standard curve

According to the concentration of the standard tube (x, mg/mL) and the absorbance ΔA standard (y, ΔA standard), establish a standard curve. From the standard curve, plug ΔA into the equation to get x (mg/mL).

2. Calculate by sample weight

(1) Calculated by sample weight

Root Vitality [μg / (g·h)]=$x \times V_{\text{Reagent II}} \times 10^3 \div (W \times T) = 666.67 \times x \div W$

10^3：Unit Conversion，1mg/mL=10^3μg /mL；$V_{\text{Reagent II}}$：Reagent II Volume，2mL；W：sample weight，g；T：Reaction time，3h。

Note:

1.The order of adding Reagent III, Reagent IV and the sample in the EP tube cannot be changed.

2.If the ΔA is not within the range of the standard curve, you can reduce the sample mass (or reduce the reaction time of the first step) or increase the sample mass (or increase the reaction time of the first step).

3.The test should be completed within 20 min after color development .

Experimental example:

1.Take 0.2081g of bean sprout roots, wash, wipe dry, operate according to the determination procedure, and measure to calculate ΔAt = A1t - A2t = 1.449-1.328=0.121, ΔAc = A1c- A2c= 1.555-1.487=0.068, ΔA=ΔAt-ΔAc=0.121-0.068=0.053, the standard curve was y=3.2172x+0.0031, which was calculated as x=0.016mg/mL, brought into the equation to calculate.

Root vitality [μg / (g-h)] = 666.67×x÷W = 51.25 μg / (g·h)

2. Take 0.2031 g of roots of sedum, washed, dried and operated according to the assay procedure and measured to calculate ΔAt= A1t- A2t= 1.517-1.424=0.093, ΔAc = A1c - A2c =1.555-1.487=0.068, ΔA =ΔAt-ΔAc=0.093-0.068=0.025, the standard curve was y=3.2172x+0.0031, which was calculated as x=0.007mg/mL,brought into the equation to calculate.

Root viability [μg / (g-h)] = 1666.67×x÷W = 22.98 μg / (g-h)

Related products:

NA0643/NA0401 Plant Dehydrogenase (PDHA) Activity Assay Kit

NA0161/NA0160 Plant Root Vitality Assay Kit(TTC method)