Plant Root Vitality Assay Kit(TTC method)

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: NA0160

Size:100T/48S

Components:

Reagent I A: powder×2. Storage at 2-8 ℃.

Reagent I B: powder×2. Storage at 2-8 ℃. Before use, take a bottle of reagent I B and add it to a bottle of reagent I A, and add 30mL of reagent II to dissolve. The reagents were prepared before the experiment. After preparation, the reagent should be stored at 2-8°C and used within a week. If the reagent turns red, it cannot be used.

Reagent II: 125mL×1. Storage at 2-8 ℃.

Reagent III: powder×3. Storage at room temperature.

Reagent IV: 120 mL×1. Storage at 2-8 ℃. Ethyl acetate, self-provided reagents. Provide a 125 mL brown bottle .

Standard: powder×1, storage at 2-8℃. Before use, add 1 mL of reagent II, shake and mix well, (10 mg/mL TTC standard solution). The reagent can be stored at 2-8°C for 1 week, if it appears red, it cannot be used .

Product Description:

The root is the main organ for plants to absorb water and mineral nutrients, and it is also the organ for the synthesis, assimilation and transformation of important substances in plants such as amino acids and hormones. Therefore, the growth and activity of roots directly affect the growth of individual plants. Nutrient level and yield level, etc. Root vigor has important practical significance.

TTC can be reduced to insoluble red triphenylformazan (TTF) by hydrogen, and TTF has an absorption peak at 485 nm. When the TTC solution penetrates into the root tissue of the plant, the reducing substances produced by the respiration process can reduce it to TTF (red), and the root tissue of the plant is stained red. The root vigor can be expressed by the reduction amount of TTC. The root activity detected by this method is the dehydrogenase activity of plant roots.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, centrifuge, water bath/incubator, adjustable pipette, micro glass cuvettes/96-well plates (non-polystyrene), mortar/homogenizer, ethyl acetate, ice and distilled water.

Procedure

I. Extraction of crude enzyme solution:

Preparation of samples: Wash the root tissue, remove the soil on the root, and dry it gently. Do not squeeze too much to damage the root cells.

II. Determination procedure

a. Preheat the spectrophotometer 30 minutes, adjust wavelength to 485nm, set zero with ethyl acetate .

b. Dilution of standard solution:

(1) Before use, take 10 μL of 10 mg/mL TTC standard solution, add 1990 μL of reagent II, mix well, (50 μg/mL TTC standard solution). (Subsequent experiments require 1000 μL. In order to reduce the experimental error, a large volume is prepared.)

(2) Take 1mL of 50 μg/mL TTC standard solution and add it to a reagent III, fully shake and mix for 2min. After mixing, 1 mL of ethyl acetate was added, and the mixture was fully shaken and mixed for 2 min. The mixture was allowed to stand at room temperature for 5 min.

(3) Dilute the upper layer solution with ethyl acetate to obtain a 20 μg/mL standard solution for use (draw 200 μL of 50 μg/mL TTC and add 300 μL of ethyl acetate to mix well).

c. Determination of standard solutions .

Take 200 μL of 20 μg/mL standard solution and 200 μL of ethyl acetate (0 μg/mL) in micro glass cuvettes/96-well plates (non-polystyrene), and measure their absorbance at 485 nm respectively.

Calculate ΔAs = A(20 μg/mL)-A(0 μg/mL). The ΔA standard only needs to be done 1-2 times.

d. Then operate according to the following table.

Reagent name(μL)	Test tube(T)	Control tube(C)
sample	0.1g	0.1g
Reagent I	1000	-
Reagent II	-	1000
All plant roots need to be immersed in the solution, react **in the dark** at 37°C for 4 hours, take out the ice bath for 5 minutes immediately, remove the filtrate, use filter paper to absorb the root water as much as possible, and place it in a mortar/homogenizer.		
Reagent IV	1000	1000

After fully grinding (it is recommended to operate in a fume hood), transfer all to a centrifuge tube, centrifuge at 12,000 rpm/min, 4 °C for 10 min, take 1 mL of supernatant into a glass cuvette, and measure the absorbance at 485 nm. Calculate ΔA= At - Ac(one control tube for each assay tube). The assay range for the ΔA assay was between 0.005-1.5.

III. Calculation formula

Calculation of root vigor according to sample mass: Root vigor is represented by the reduction amount of TTC.

TTC reduction strength [μg TTC/(g h)] = ΔA × Cs÷ ΔAs × V÷ (W × T) = 5 × ΔAt ÷ ΔAs ÷ W

W: root weight, g; Cs: standard solution concentration, 20 μg/mL; T: reaction time, 4 h; V: volume of reagent IV , volume of homogenate, 1 mL.

Note:

1. Reagent IV is volatile and toxic. For health, please wear lab coat, mask and latex gloves for operation.
2. If the dark reaction of the sample at 37°C has not reached 4 hours but the root has appeared deep pink, the next experimental operation can be directly carried out at this time; if the ΔA is greater than 1.5, the sample

quality can be reduced or the reaction time can be shortened for the experiment, and the calculation formula should be modified.

3. If the root does not appear pink or the ΔAt is less than 0.005 after the 4h dark reaction, you can extend the dark reaction time (8h, 16h or even 24h) or increase the sample, and the calculation formula should be modified.
4. If the supernatant to be tested is still turbid after centrifugation, try increasing the centrifugation speed or prolonging the time, such as centrifuging at 15000rpm and 4°C for 20min.

Experimental example:

1. Weigh the roots of 0.131g of onions, wash, dry, and operate according to the measurement steps. Use a 96-well plate (non-polystyrene material) to measure and calculate ΔAt=At-Ac=0.714-0.082=0.632, ΔAs= A(20μg/mL)-A(0μg/mL)=0.660-0.042=0.618, bring into the formula to calculate:

TTC reduction strength = 5 × ΔAt ÷ ΔAs ÷ W = 39.033 μg TTC/(g h)

2. Weigh 0.126g of the root of Sedum, wash, dry, and operate according to the measurement steps. Use a 96-well plate (non-polystyrene material) to measure and calculate ΔAt= At-Ac=0.422-0.139=0.283 , ΔAs= A(20μg/mL)-A(0μg/mL)=0.660-0.042=0.618, bring into the formula to calculate:

TTC reduction strength = 5×ΔAt÷ ΔAs÷ W = 18.172 μg TTC/(g·h)

3. Weigh the root of 0.118g garlic, wash it, wipe it dry, follow the measurement steps, use a 96-well plate (non-polystyrene material) to measure and calculate ΔAt= At- Ac= 0.191-0.055 = 0.136, ΔAs= A(20μg/mL)-A(0μg/mL)=0.660-0.042=0.618, bring into the formula to calculate:

TTC reduction strength = 5×ΔAt÷ ΔAs÷ W = 9.325 μg TTC/(g·h)

References:

[1] Xiuyun Zhu, Meng Liang,Yu Ma. A Review Report on the Experiments for the Determination of Root Activity by TTC Method[J]. Guangdong Chemical Industr, 2020.

[2] Sangen Wang. Plant Physiology Experiment Course[M]. Science Press, 2005.

Related products:

NA0643/NA0401 Plant Dehydrogenase (PDHA) Activity Assay Kit