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Soil Superoxide Dismutase(S-SOD)Activity Assay Kit(WST Method)

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Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: AK1035-100T-48S

Size: 100T/48S

Product Description: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact SunLong staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Reagent I	Liquid 5 mL×1	2-8℃
Reagent II	Liquid 25 μL×1	2-8℃
Reagent III	Liquid 4 mL×1	2-8℃
Reagent IV	Liquid 0.12 mL×1	2-8℃

Solution Preparation:

1. Reagent II working solution: Before using Reagent II, use a handheld centrifuge to spin the tube until the contents are at the bottom, then mix well by pipetting up and down. Before use, prepare the working solution according to the sample volume, using a ratio of Reagent II to distilled water of 5 μL:245 μL (total 250 μL, about 12 T). Use immediately.

2. Reagent IV Working solution: Before using Reagent IV, use a handheld centrifuge to spin the tube until the contents are at the bottom. Before use, prepare the working solution according to the sample volume, using a ratio of Reagent IV to distilled water of 20 μL:180 μL (total 200 μL, about 20T). The solution is ready for use.

Product Description:

Soil superoxide dismutase (S-SOD) plays an important role in soil ecosystems by catalyzing the disproportionation of superoxide anions to produce H_2O_2 and O_2. Thus, SOD not only acts as a superoxide

anion scavenger but also serves as a major H_2O_2-producing enzyme, playing a key role in biological antioxidant systems. Superoxide anions (O_2^-) are generated by the xanthine–xanthine oxidase system and can react with WST-1 to form a water-soluble yellow formazan product with an absorption peak at 450 nm. SOD scavenges O_2^-, thereby inhibiting formazan formation. Therefore, the darker the yellow color of the reaction solution, the lower the SOD activity, and vice versa.

Reagents and Equipment Required but Not Provided:

Visible spectrophotometer/microplate reader, benchtop centrifuge, water bath/thermostatic incubator, electronic balance, adjustable pipette, microglass cuvette/96-well plate, ultrasonic disruptor, ice, and distilled water.

Operation procedure

I. Sample preparation: (The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Fresh soil samples are naturally air-dried or air-dried in an oven at 37°C, and sieved through 30~50 mesh.

2. According to the ratio of air-dried soil sample (g): Extract solution volume (mL) is 1~2:5 (it is recommended to weigh about 0.2g air-dried soil sample, add 1mL Extract solution), ultrasonic crushing extraction (ice bath, power 200W, ultrasonic 3s, interval 10s, total 10min), 8000g centrifuge at 4°C for 10 min, take the supernatant, and place it on ice for testing.

II. Determination procedure:

1. The spectrophotometer/microplate reader is preheated for more than 30 minutes, the wavelength is adjusted to 450nm, and the distilled water of the spectrophotometer is adjusted to zero.

2. Before the measurement, take part according to the sample volume and put Reagent I, Reagent III, Reagent IV working solution, (distilled water) in a water bath at 37°C for more than 5 minutes.

3. Loading table (add the following reagents to 1.5 mL EP tubes or 96-well plates in order)

Reagent Name (μL)
Test tube(T)
Control tube(C)
Blank tube1(B1)
Blank tube2(B2)
Sample
90
90
-
-
Reagent I
45
45
45
45
Reagent II working solution
20

-
20
-
Reagent III
35
35
35
35
Distilled water
-
20
90
110
Reagent IV working solution
10
10
10
10

After thorough mixing and 37°C water bath for 30 minutes, the absorbance value A of each tube was measured at 450nm. It is recorded as AT, AC, AB1, and AB2, and ΔAT = AT - AC, ΔAB = AB1 - AB2. (Blank tube 1 and blank tube 2 only need to make 1~2 pipes each; One control tube per sample).

III. Calculation of SOD activity

1. Calculation of suppression percentage:

Percentage of inhibition = (ΔAB - ΔAT) ÷ ΔAB × 100%

Try to keep the suppression percentage of the sample in the range of 30–70%, and the closer it is to 50%, the more accurate it is. If the calculated percentage of inhibition is less than 30% or greater than 70%, it is usually necessary to adjust the amount and redetermine. If the measured percentage of inhibition is high, the sample needs to be diluted appropriately; if the measured percentage of inhibition is low, it is recommended to increase the soil sample mass in sample preparation.

2. SOD enzyme activity unit: When the inhibition percentage is 50% in the xanthine oxidase coupling reaction system, the SOD enzyme activity in the reaction system is defined as an enzyme activity unit.

3. Calculation of SOD enzyme activity:

SOD activity (U/g mass) = [inhibition percentage÷ (1-inhibition percentage) ×VRT]÷ (W×VS÷VE)×F

=2.222× Suppression Percentage÷(1-Suppression Percentage) ÷W×F

VRT: total volume of the reaction system, 0.2mL;

VS: The volume of the sample added to the reaction system, 0.09mL；

VE: add extraction liquid volume, 1mL;

W: Sample mass, g;

F: Sample dilution fold.

Notes:

1. Samples and Reagent II working solution are placed on ice when used.

2. When there are many samples, the working solution of the test tube and the working solution of the control tube can be prepared according to the table (including Reagent I, (Reagent II working solution), Reagent III, (distilled water)), and the working solution of Reagent IV working solution must be added last.

Experimental example:

Take 0.2039g of air-dried soil sample and add 1mL of Extract solution for ultrasound, follow the measurement steps, and use a 96-well plate to calculate：ΔAT = AT-AC= 0.687 - 0.128= 0.559, ΔAB=ΔAB1-ΔAB2=1.142-0.054=1.088, inhibition percentage=(ΔAB-ΔAT) ÷ΔAB×100%=48.62%, and the enzyme activity was calculated by sample mass:

SOD activity (U/g mass) = 2.222× inhibition percentage÷ (1-inhibition percentage) ÷W×F =10.31 U/g mass.