Soil Dehydrogenase Assay Kit (Visible Spectrophotometry)

Description

Soil dehydrogenase (S-DHA) activity reflects the biomass of active microorganisms in soil systems and their capacity for organic matter degradation, and can therefore be used as an indicator of the degradative activity of soil microorganisms.

Detection Principle

The hydrogen acceptor 2,3,5-triphenyl tetrazolium chloride (TTC) accepts hydrogen generated during cellular respiration and is subsequently reduced to triphenyl formazan (TF). TF is red in color and exhibits a maximum absorption peak at 485 nm. The absorbance at 485 nm is measured by visible spectrophotometry to determine soil dehydrogenase activity.

Specifications

Composition and Storage Conditions (50T/24S):

Component	Specification	Storage Condition
CB0154V-A	Powder × 1 vial	Before use, dissolve in 30 mL of distilled water. Prepare freshly whenever possible. Store at 4°C protected from light.
CB0154V-B	50 mL × 1 bottle	Store at 4°C
CB0154V-C	Acetone	Self-provided

Note: Before formal measurement, it is recommended to select 2–3 samples with expected large differences for a preliminary assay.

Instructions

I. Required Equipment and Materials:

Visible spectrophotometer, 1 mL glass cuvette, 30–50 mesh sieve, analytical balance, constant-temperature incubator or water bath, refrigerated centrifuge, ice, distilled water, and acetone (cannot be shipped and must be provided by the user).

II. Sample Preparation:

1. Soil Samples

Accurately weigh approximately 0.1 g of fresh soil sample that has been passed through a 30–50 mesh sieve (to ensure sufficient contact between TTC and soil particles).

2. Sludge Samples

Wash the sludge sample with distilled water, centrifuge at 10,000 × g and 25°C for 10 min, discard the supernatant, and repeat the washing procedure 3–4 times.

III. Assay Procedure:

1. Preheat the spectrophotometer for 30 min, set the wavelength to 485 nm, and zero the instrument with distilled water.

2. Add the following reagents sequentially into EP tubes:

Reagent	Control Tube (μL)	Sample Tube (μL)
Sample (g)	0.1g	0.1g
CB0154V-A		500
CB0154V-B	1000	500
Mix thoroughly and incubate at 37°C in the dark for 6 h. Immediately place the tubes in an ice bath for 5 min.		
CB0154V-C	500	500
Vortex several times and incubate at 37°C for 10 min. Centrifuge at 10,000 × g and 4°C for 5 min. Transfer 1 mL of the supernatant into a 1 mL glass cuvette and measure the absorbance of the Control Tube and Sample Tube at 485 nm, recorded as A_control and A_sample, respectively. Calculate: ΔA = A_sample − A_control Note: One Control Tube should be prepared for each Sample Tube.		

IV. Calculation of S-DHA Activity:

Definition of Enzyme Activity Unit: One unit of enzyme activity is defined as the amount of enzyme that increases the OD at 485 nm by 0.01 per hour per gram of sample in 1 mL of reaction system at 37°C.

Calculation Formula: S-DHA Activity (U/g) = (ΔA÷0.01)÷T÷W = 166.7×ΔA

Note: T: Reaction time, 6 h; W: Sample weight, 0.1 g

Precautions

1. The prepared CB0154V-A should be stored at 4°C protected from light and is recommended for use within one week. If the solution turns red, it should not be used.
2. Immediately place the reaction mixture in an ice bath after completion of the reaction to terminate the reaction and completely remove any residual reaction solution.
3. If the measured absorbance is too high, reduce the amount of sample and repeat the assay. If the absorbance is too low, extend the incubation time and modify the calculation formula accordingly.
4. If the supernatant remains turbid after centrifugation, increasing the centrifugation speed or extending the centrifugation time may be attempted.
5. This product is intended for scientific research use only by qualified professionals. It must not be used for clinical diagnosis or treatment, food or pharmaceutical applications, and must not be stored in residential environments.
6. CB0154V-C (acetone) is volatile and toxic. For your safety and health, please wear a lab coat, mask, and latex gloves during operation.