Solid-Cellulase(S-CL) Activity Assay Kit

Product Code: 67126

Soil Cellulase (S-CL) Activity Assay Kit

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Method: Spectrophotometric method

Package size: 50T

Product Introduction

Soil cellulase (S-CL) is mainly derived from soil microorganisms. S-CL catalyzes crop straw cellulose to produce glucose, an important carbon-source nutrient.

This kit uses the 3,5-dinitrosalicylic acid method to determine S-CL activity by measuring the reducing sugars produced during catalyzed cellulose degradation.

Sample example: lawn soil. Actual readings may vary depending on testing conditions and instruments.

Package List and Storage

Item Code	Component	Specification	Storage
67126.1	Reagent I	Self-provided	/
67126.2	Reagent II	15 mL	2–8°C
67126.3	Reagent III	60 mL	2–8°C
67126.4	Reagent IV	10 mL	2–8°C, protected from light
67126.5	Standard	10 mg	2–8°C
67126.m	Manual	1 copy	/

Quality and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent I	——	——
Reagent II	——	——
Reagent III	——	——
Reagent IV	——	——
Standard	——	——

Transport and Storage

Transport: This product is transported with ice packs.

Storage: Store according to the instructions. Shelf life is 180 days.

Instructions for Use

1. Sample Processing

Air-dry fresh soil samples naturally or oven-dry at 37°C. Pass the dried samples through a 30–50 mesh sieve.

2. Reagent Preparation

- **Reagent I:** Toluene, 7 mL × 1 bottle, stored at 4°C. This reagent is self-prepared.
- **Standard:** 10 mg anhydrous glucose. Before use, add 1 mL distilled water to dissolve and prepare a 10 mg/mL glucose solution. Store at 2–8°C for up to two weeks. For longer storage, dissolve with saturated benzoic acid solution.

3. Assay Procedure

1. Preheat the visible spectrophotometer or microplate reader for 30 min. Set the wavelength to 540 nm and zero with distilled water.
2. Dilute the 10 mg/mL standard solution with distilled water to prepare 1, 0.8, 0.6, 0.4, and 0.2 mg/mL standard solutions for testing.

Standard Solution Dilution

No.	Concentration Before Dilution (mg/mL)	Standard Solution Volume (μL)	Distilled Water Volume (μL)	Concentration After Dilution (mg/mL)
1	10	100	900	1
2	1	160	40	0.8
3	1	120	80	0.6
4	1	80	120	0.4
5	1	40	160	0.2

In the following experiments, each standard tube requires 50 μL standard solution. Do not directly measure absorbance at this dilution step.

Assay Operation

Component or Step	Control Tube	Assay Tube	Standard Tube	Blank Tube
Air-dried soil sample	0.25 g	0.25 g	-	-
Reagent I	125 μL	125 μL	-	-
Control tube treatment	Boil for 15 min. Wrap with sealing film to prevent the cap from popping off.	-	-	-
Assay tube treatment	-	Shake to mix thoroughly and place at room temperature for 15 min.	-	-
Reagent II	250 μL	250 μL	-	-
Reagent III	1000 μL	1000 μL	-	-
Distilled water	250 μL	250 μL	-	-
Saccharification	Shake and mix well. Incubate in a 40°C water bath for 1 h, then boil for 15 min. Wrap with sealing film to prevent the cap from popping off. Cool, centrifuge at 10000 rpm at room temperature for 10 min, and collect the supernatant as the saccharified solution.	Shake and mix well. Incubate in a 40°C water bath for 1 h, then boil for 15 min. Wrap with sealing film to prevent the cap from popping off. Cool, centrifuge at 10000 rpm at room temperature for 10 min, and collect the supernatant as the saccharified solution.	-	-
Saccharified solution	50 μL	50 μL	-	-
Standard	-	-	50 μL	-
Distilled water	-	-	-	50 μL
Reagent IV	150 μL	150 μL	150 μL	150 μL
Color development	Mix well and boil in a boiling water bath for 15 min. Wrap with sealing film to prevent the cap from popping off. Cool.	Mix well and boil in a boiling water bath for 15 min. Wrap with sealing film to prevent the cap from popping off. Cool.	Mix well and boil in a boiling water bath for 15 min. Wrap with sealing film to prevent the cap from popping off. Cool.	Mix well and boil in a boiling water bath for 15 min. Wrap with sealing film to prevent the cap from popping off. Cool.
Distilled water	1050 μL	1050 μL	1050 μL	1050 μL

Mix well after cooling. Transfer 1000 μL into a 1 mL glass cuvette and measure absorbance at 540 nm. Record the values as $A_{control}$, $A_{measured}$, $A_{standard}$, and A_{blank}.

Calculate $\Delta A_{measured} = A_{measured} - A_{control}$ and $\Delta A_{standard} = A_{standard} - A_{blank}$.

> For each assay tube, set up one control tube. Blank tubes and the standard curve only need to be run 1–2 times.

Activity Calculation

1. Standard Curve

Use the standard tube concentration X (mg/mL) and absorbance $\Delta A_{standard}$(Y) to establish a standard curve. Substitute $\Delta A_{measured}$ into the standard curve to calculate the sample concentration X (mg/mL).

2. S-CL Enzyme Activity

Unit definition: The amount of enzyme in each g soil sample that produces 1 mg glucose per day is defined as one enzyme activity unit.

S-CL enzyme activity (U/g soil sample) = $X \times V_{total\ reaction} \div W \div T = 156 \times X$

- **X:** Sample concentration calculated from the standard curve, mg/mL
- **T:** Reaction time, 1 h = 1/24 d
- **$V_{total\ reaction}$:** Total volume of the reaction system, 1.625 mL
- **W:** Sample mass, 0.25 g

Precautions

1. This 50T kit can test 24 samples. Before formal measurement, it is recommended to select 2–3 samples with large expected differences for a preliminary test.
2. Instruments and supplies required but not provided: visible spectrophotometer, water bath or metal bath, adjustable pipette, 1 mL glass cuvette, 30–50 mesh sieve, toluene, and distilled water.
3. If the absorbance of the sample assay tube is too low, 0.01, the reaction time can be extended by increasing the 40°C water-bath saccharification time, possibly to 24 h or longer. The formula must be adjusted accordingly during calculation.
4. Alternatively, the saccharified solution volume in the color development step can be adjusted while reducing the distilled water volume. In some cases, the distilled water volume may be completely replaced by saccharified solution. The standard curve must be modified accordingly.