**Soil Cellulase (S-CL) Activity Assay Kit - Micro Method**

**Product code:** 67127

**Size:** 100T

## Product Introduction

Soil cellulase (S-CL) is mainly derived from soil microorganisms. S-CL catalyzes the degradation of 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 based on reducing sugars produced during catalyzed cellulose degradation.

Sample example: lawn soil. Actual readings may vary under different testing conditions and with different instruments.

## Package Contents and Storage

| Item Code | Component | Amount | Storage |
|---|---|---|---|
| 67127.1 | Reagent 1 | Self-prepared | / |
| 67127.2 | Reagent 2 | 6 mL | 2–8°C |
| 67127.3 | Reagent 3 | 25 mL | 2–8°C |
| 67127.4 | Reagent 4 | 3.5 mL | 2–8°C, protect from light |
| 67127.4 | Standard | 10 mg | 2–8°C |
| 67127.m | Manual | 1 copy | / |

## Quality and Safety Information

| Component | Quality Standard | Main Toxicity |
|---|---|---|
| Reagent 1 | — | — |
| Reagent 2 | — | — |
| Reagent 3 | — | — |
| Reagent 4 | — | — |
| Standard | — | — |

## Transportation and Storage

- **Transportation:** Transport with ice packs.
- **Storage:** Store according to the component instructions.
- **Shelf life:** 180 days.

## Instructions for Use

### 1. Sample Processing

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

### 2. Reagent Preparation

- **Reagent I:** Toluene, 3 mL × 1 bottle, user-supplied.
- **Standard:** Add 1 mL distilled water to 10 mg anhydrous glucose before use to prepare a 10 mg/mL glucose solution. Store at 2–8°C. The solution can be stored for two weeks. If dissolved with saturated benzoic acid solution, it can be stored for a longer time.

## 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 experiment, each standard tube requires 10 μL of standard solution. Do not measure the absorbance directly at this step.

### Assay Operation

| Component or Step | Control Tube | Assay Tube | Standard Tube | Blank Tube |
|---|---|---|---|---|
| Air-dried soil sample | 0.05 g | 0.05 g | — | — |
| Reagent I | 25 μL | 25 μL | — | — |
| Control pretreatment | Boil for 15 min. Wrap with sealing film to prevent the cap from popping off. | Shake and mix thoroughly; let stand at room temperature for 15 min. | — | — |
| Reagent II | 50 μL | 50 μL | — | — |
| Reagent III | 200 μL | 200 μL | — | — |
| Distilled water | 50 μL | 50 μL | — | — |
| Saccharification | Vortex to mix thoroughly. Incubate in a 40°C water bath for 1 h. Boil for 15 min, wrapping with sealing film to prevent the cap from popping off. Cool, centrifuge at 10,000 rpm at room temperature for 10 min, and collect the supernatant as the saccharification solution. | Same as control tube. | — | — |
| Saccharification solution | 10 μL | 10 μL | — | — |
| Standard | — | — | 10 μL | — |
| Distilled water | — | — | — | 10 μL |
| Reagent IV | 30 μL | 30 μL | 30 μL | 30 μL |
| Color development | Mix thoroughly, then boil in a boiling water bath for 15 min. Wrap with sealing film to prevent the cap from popping off. Cool. | Same as control tube. | Same as control tube. | Same as control tube. |
| Distilled water | 210 μL | 210 μL | 210 μL | 210 μL |
| Measurement | Mix thoroughly. After cooling, transfer 200 μL to a micro glass cuvette or 96-well plate and measure $A_{control}$ at 540 nm. | Measure $A_{assay}$ at 540 nm. | Measure $A_{standard}$ at 540 nm. | Measure $A_{blank}$ at 540 nm. |

Calculate the absorbance values as follows:

- $\Delta A_{assay} = A_{assay} - A_{control}$
- $\Delta A_{standard} = A_{standard} - A_{blank}$

Set up one control tube for each assay tube. Blank tubes and the standard curve only need to be performed 1–2 times.

## Activity Calculation

### 1. Standard Curve

Use the standard tube concentration, X in mg/mL, and the corresponding absorbance, $Y = \Delta A_{standard}$, to establish the standard curve. Substitute the sample ΔA value, $Y = \Delta A_{assay}$, into the standard curve formula to calculate the sample concentration, X in mg/mL.

### 2. S-CL Enzyme Activity

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

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

- **T:** reaction time, 1 h = 1/24 d
- **$V_{total\ reaction}$:** total volume of the reaction system, 0.325 mL
- **W:** sample mass, 0.05 g

## Precautions

1. This 100T kit can test 48 samples. Before formal measurement, it is recommended to select 2–3 samples with large expected differences for a preliminary test.
2. User-prepared instruments and supplies: visible spectrophotometer or microplate reader, water bath or metal bath, adjustable pipettes, micro glass cuvettes or 96-well plate, 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. This refers to the 40°C water-bath saccharification time, which may be extended to 24 h or longer. The formula must be adjusted accordingly during calculation.
4. The volume of saccharification solution used in the color development step may also be adjusted while reducing the volume of distilled water. In some cases, the entire distilled water volume may be replaced by saccharification solution. The standard curve must be modified accordingly.