

## Soil Cellulase (S-CL) Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer/ Microplate reader

**Catalog Number:** BC0155

**Size:**100T/48S

**Product Composition:** 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 Solarbio staff in time.

Reagent name	Size	Preservation Condition
Reagent I	Self-Provided Reagent	-
Reagent II	Liquid 5 mL×1	2-8°C
Reagent III	Liquid 20 mL×1	2-8°C
Reagent IV	Liquid 6 mL×1	2-8°C
Standard	Powder ×1	2-8°C

### Solution Preparation:

1. Reagent I: About 3mL methylbenzene (Required but not provided), store at RT. A 30mL brown reagent bottle is provided in the kit. Please label the reagent name yourself.
2. Standard: Contain 10 mg of anhydrous glucose (dry weight loss < 0.2%). Dissolve the standard with 1 mL of distilled water to generate a 10 mg/mL glucose solution standard, store at 2-8°C and use within two weeks or dissolve the standard with saturated benzoic acid solution stored for a longer time.

### Product Description

Soil Cellulase (S-CL) mainly comes from soil microorganisms. Glucose produced by S-CL is the main carbon source nutrients of soil microorganisms. In this kit, this product uses the 3,5-dinitrosalicylic acid method to determine the content of reducing sugars produced by S-CL catalyzing cellulose degradation.

### Reagents and Equipment Required but Not Provided.

Spectrophotometer/microplate reader, water-bath, transferpettor, 30-50 mesh sieve, mortar, micro glass cuvette/96 well flat-bottom plate, methylbenzene (>98%, AR), ice and distilled water.

### Procedure:

#### I. Sample processing:

Fresh soil samples are naturally air-dried or oven to dry at 37°C, then sieved by 30-50 mesh sieve.

#### II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 540 nm, and set spectrophotometer zero with distilled water.
2. Standard preparation: Dilute the standard to 1, 0.8, 0.6, 0.4, 0.2, 0.1 mg/mL with distilled water.

3. Add the following reagents to the EP tube in turn:

Reagent	Contrast Tube (C)	Test Tube (T)	Standard Tube (S)	Blank Tube (B)
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Water-free soil (g)	0.05	0.05	-	-
Reagent I (μL)	25	25	-	-
	Boil for 15 minutes (Wrap the sealing film to prevent bursting)	Shack to mix thoroughly, place at RT for 15 minutes.	-	-
Reagent II (μL)	45	45	-	-
Reagent III (μL)	185	185	-	-
Distilled water (μL)	45	45	-	-
Shake to mix thoroughly, then saccharification in water bath at 40°C for 1 hour. After the saccharification, boil for 15 minutes (Wrap the sealing film to prevent bursting), 10000rpm for 10min at RT and take the supernatant (saccharification liquid).				
Saccharified liquid (μL)	15	15	-	-
Standard solution (μL)	-	-	15	-
Distilled water (μL)	-	-	-	15
Reagent IV (μL)	35	35	35	35
Mix thoroughly, boil in boiling water bath for 15 minutes (Wrap the sealing film to prevent bursting), then leave the tube to cool.				
Distilled water (μL)	250	250	250	250
Mix thoroughly. After cooling, take 200 μL to micro glass cuvette/96 well flat-bottom plate, then detect the absorbance at 540 nm and noted as A <sub>C</sub> , A <sub>T</sub> , A <sub>S</sub> , and A <sub>B</sub> . $\Delta A_T = A_T - A_C$ . $\Delta A_S = A_S - A_B$ . The blank tube and standard curve only need to be measured 1-2 times. A contrast tube is required for each test tube.				

### III. Calculation:

#### 1. Standard curve

The concentration of standard solution as x-axis,  $\Delta A_S$  as y-axis, obtain the equation  $y=kx+b$ . Take  $\Delta A_T$  to the equation to acquire x value (mg/mL).

#### 2. Calculation

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 milligram of glucose in the reaction system per day every gram soil sample.

$$S\text{-CL activity (U/g weight)} = x \times V_r \div W \div T = 144 \times x$$

T: Reaction time, 1 hour=1/24 day;

V<sub>r</sub>: Total reaction volume, 0.3 mL;

W: Sample weight, 0.05 g.

#### Note:

If the absorbance of sample tube is too small (0.01), prolong the reaction time at 40°C (24h or more)

or increase the adding volume of saccharification liquid in the coloration step while decrease the volume of distilled water correspondingly. And modify the calculation formula.

### Recent Product Citations:

- [1] Xiao X, Li J, Lyu J, Feng Z, Zhang G, Yang H, Gao C, Jin L, Yu J. Chemical fertilizer reduction combined with bio-organic fertilizers increases cauliflower yield via regulation of soil biochemical properties and bacterial communities in Northwest China. *Front Microbiol.* 2022 Jul 27;13:922149. doi: 10.3389/fmicb.2022.922149. PMID: 35966650; PMCID: PMC9363920.
- [2] Muhammad I, Yang L, Ahmad S, Zeeshan M, Farooq S, Ali I, Khan A, Zhou XB. Irrigation and Nitrogen Fertilization Alter Soil Bacterial Communities, Soil Enzyme Activities, and Nutrient Availability in Maize Crop. *Front Microbiol.* 2022 Feb 3;13:833758. doi: 10.3389/fmicb.2022.833758. PMID: 35185852; PMCID: PMC8851207.
- [3] Pu Q, Zhang K, Poulain AJ, Liu J, Zhang R, Abdelhafiz MA, Meng B, Feng X. Mercury drives microbial community assembly and ecosystem multifunctionality across a Hg contamination gradient in rice paddies. *J Hazard Mater.* 2022 Aug 5;435:129055. doi: 10.1016/j.jhazmat.2022.129055. Epub 2022 May 4. PMID: 35650726.
- [4] Yang L, Muhammad I, Chi YX, Wang D, Zhou XB. Straw Return and Nitrogen Fertilization to Maize Regulate Soil Properties, Microbial Community, and Enzyme Activities Under a Dual Cropping System. *Front Microbiol.* 2022 Mar 15;13:823963. doi: 10.3389/fmicb.2022.823963. PMID: 35369510; PMCID: PMC8965350.
- [5] Song K, Zhou Z, Leng J, Fang S, Zhou C, Ni G, Kang L, Yin X. Effects of rumen microorganisms on the decomposition of recycled straw residue. *J Zhejiang Univ Sci B.* 2023 Apr 15;24(4):336-344. doi: 10.1631/jzus.B2200504. PMID: 37056209; PMCID: PMC10106401.

### References:

- [1] Deng S P, Tabatabai M A. Cellulase activity of soils[J]. *Soil Biology and Biochemistry*, 1994, 26(10): 1347-1354.
- [2] Sinegani A A S, Sinegani M S. The effects of carbonates removal on adsorption, immobilization and activity of cellulase in a calcareous soil[J]. *Geoderma*, 2012, 173: 145-151.

### Related Products:

BC0280/BC0285	Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit
BC0110/BC0115	Soil Polyphenoloxidase (S-PPO) Activity Assay Kit
BC0120/BC0125	Soil Urease(S-UE) Activity Assay Kit
BC0140/BC0145	Soil Acid Phosphatase(S-ACP) Activity Assay Kit