Solid-Cellulase(S-CL) Activity Assay Kit

Product Code: 67128

Solid-Cellulase (S-CL) Activity Assay Kit, Microplate Method

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This kit measures soil cellulase (S-CL) activity. S-CL is mainly derived from soil microorganisms and catalyzes cellulose in crop straw to produce glucose, an important carbon source nutrient. The assay uses the 3,5-dinitrosalicylic acid method to determine S-CL activity based on the reducing sugars produced during cellulose degradation.

Package Contents and Storage

Code	Item	Quantity	Storage
67128.1	Reagent I	Self-prepared	/
67128.2	Reagent II	6 mL	2-8°C
67128.3	Reagent III	25 mL	2-8°C
67128.4	Reagent IV	3.5 mL	2-8°C, protected from light
67128.5	Standard	10 mg	2-8°C
67128.m	Instructions	1 copy	/

Quality and Safety Information

Material	Quality Standard	Main Toxicity
Reagent I	--	--
Reagent II	--	--
Reagent III	--	--
Reagent IV	--	--
Standard	--	--

Transportation and Storage

Transportation: Transport with ice packs.

Storage: Store according to the conditions listed above. Shelf life is 180 days.

Materials Required but Not Provided

- Microplate reader
- Water bath or metal bath
- Adjustable pipettor
- 96-well plate
- 30-50 mesh sieve
- Toluene
- Distilled water

Instructions for Use

1. Sample Processing

Naturally air-dry fresh soil samples, or dry them in a 37°C oven. Pass the dried samples through a 30-50 mesh sieve.

2. Reagent Preparation

- **Reagent I:** Toluene, 3 mL × 1 bottle. Prepare by the user.
- **Standard:** Add 1 mL distilled water to 10 mg anhydrous glucose and dissolve to prepare a 10 mg/mL glucose solution. Store at 2-8°C for up to two weeks, or dissolve with saturated benzoic acid solution for longer storage.

3. Assay Procedure

1. Preheat the 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.

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 standard solution. Do not measure absorbance directly at this dilution step.

Assay Setup

Component or Step	Control Tube	Assay Tube	Standard Tube	Blank Tube
Air-dried soil sample (g)	0.05	0.05	-	-
Reagent I (μL)	25	25	-	-
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 (μL)	50	50	-	-
Reagent III (μL)	200	200	-	-
Distilled water (μL)	50	50	-	-
Saccharification	Shake and mix thoroughly. Incubate in a 40°C water bath for 1 h, then boil for 15 min. Wrap with sealing film to prevent caps from popping. Cool, centrifuge at 10000 rpm at room temperature for 10 min, and collect the supernatant as the saccharification solution.		-	-
Saccharification solution (μL)	10	10	-	-
Standard (μL)	-	-	10	-
Distilled water (μL)	-	-	-	10
Reagent IV (μL)	30	30	30	30
Color development	Mix thoroughly and boil in a boiling water bath for 15 min. Wrap with sealing film to prevent caps from popping. Cool.			
Distilled water (μL)	210	210	210	210
Measurement	Mix thoroughly. After cooling, transfer 200 μL to a 96-well plate and measure absorbance at 540 nm.			

Record absorbance values as $A_{control}$, A_{assay}, $A_{standard}$, and A_{blank}.

Calculate: $\Delta A_{assay} = A_{assay} - A_{control}$

Calculate: $\Delta A_{standard} = A_{standard} - A_{blank}$

Set one control tube for each assay 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 the standard curve. Substitute ΔA_{assay}(Y) into the standard curve equation to calculate the sample concentration (X, mg/mL).

2. S-CL Enzyme Activity

Unit definition: The production of 1 mg glucose per g soil sample 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, 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. If the absorbance of the sample assay tube is too low, such as 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 changed accordingly during calculation.
3. The volume of saccharification solution used in the color development step can 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.