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# β-1,4-Glucanase / Cellobiosidase (S-C1) Activity Assay Kit

Operation Equipment: Spectrophotometer/ Microplate reader

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

Catalog Number: AK0085

**Size:** 100T/48S

#### **Components:**

Extract solution:60mL×1. Storage at 4°C.

Reagent I: Powder×1. Storage at 4°C. Add 5 mL of distilled water to fully dissolve when the solution will be used. Store unused reagents at 4°C for 2 weeks.

Reagent II:30mL×1. Storage at 4°C.

Standard solution: 1mL×1, 5µmol/mL p-nitrophenol solution. The standard is diluted 16 times with reagent III to obtain a 0.3125 µmol/mL standard solution before use.

#### **Product Description**

β-1,4-glucanase/cellobiosidase (C1, EC3.2. 1.91) exists in bacteria, fungi and animals, and is a component of the cellulase system. The end of the linear molecule hydrolyzes the β-glucosidic bond and cuts out one cellobiose molecule every time.

C1 can catalyze p-nitrobenzene cellobiose (PNPC) to p-nitrophenol, which has a characteristic light absorption at 400nm.

## Reagents and Equipment Required but Not Provided

Spectrophotometer/ microplate reader, centrifuge, water-bath, transferpettor, micro glass cuvette/ 96 well flat-bottom plate, mortar/homogenizer, and distilled water.

#### **Procedure**

#### 1. Sample Extraction:

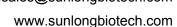
#### (1) Tissue sample:

According to the mass of the tissue (g): the volume of the extract solution (mL) is 1: 5- 10. Suggested 0. 1g of tissue with 1mL of extract solution. Fully grind on ice, centrifugate at 10000g and 4°C for 10min. Supernatant is placed on ice for test.

#### (2) Bacteria or cells:

According to the number of cells (10<sup>4</sup>): the volume of the extract solution (mL) is 500-1000: 1. Suggest 5 million with 1mL of Extract Solution. Use ultrasonication to split bacteria or cells (power 300W, work time 3s, interval 7s, total time 3 min), centrifugated at 10000g and 4°C for 10min. Supernatant is placed on ice for test.

(3) Serum/plasma: direct measurement.





#### 2. Determination steps and sample adding table:

- a. Preheat spectrophotometer/ microplate reader more than 30 min, adjust wavelength to 400 nm and set zero with distilled water.
- b. Operate according to the following table:

Reagent Name(μL)	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube (B)
Reagent I	80			
Distilled water	_	80	80	100
Standard solution	_	_	20	_
sample	20	20	-	_
Reacting for 1 h at 37°C in a water bath.			_	-
Reagent II	200	200	200	200

Mix well, react for 2 minutes at RT. Take 200 $\mu$ L react solution to micro glass cuvette or 96 well flat-bottom plate and record the absorption value a of each tube at 400 nm, calculate  $\Delta A = A_T - A_C$ ,  $\Delta A_S = A_S - A_B$ .

## Calculation of C1 activity:

#### 1) Tissue protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1  $\mu$ mol ofp-nitrophenol every mg of protein in the reaction system per hour.

C1 Activity (U/mg prot)= 
$$\Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_S \div (Cpr \times V_S) \div T = 312.5 \times \Delta A \div \Delta A_S \div Cpr$$

2) Tissue weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 µmol ofp-nitrophenol every gram of tissue in the reaction system per hour.

C1 Activity (U/g weight) = 
$$\Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_S \div (V_S \div V_E \times W) \div T = 312.5 \times \Delta A \div \Delta A_S \div W$$

3) Liquid

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1  $\mu$ mol ofp-nitrophenol every milliliter of liquid sample in the reaction system per hour.

C1 Activity (U/mL) =
$$\Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_S \div V_S \div T = 312.5 \times \Delta A \div \Delta A_S$$

4) Bacteria or cultured cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1  $\mu$ mol ofp-nitrophenol every 10<sup>4</sup> cells or bacteria in the reaction system per hour at.

C1 Activity (U/10<sup>4</sup> cell) = $\Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_S \div (V_S \div V_E \times \text{cell amount}) \div T = 312.5 \times \Delta A \div \Delta A_S \div \text{cell amount};$ 

V<sub>S</sub>: Sample volume, 0.02mL

Cs: Standard concentration, 0.3125µmol/mL

Ve: Extract solution volume, 1 mL;

Cpr: Supernatant sample protein concentration (mg/mL);

T: Reaction time (min), 1 hour;

W: Sample weight, g;





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Cell amount: 10 thousand as unit.

#### Note

1. If the absorbance value is greater than 1.5, it is recommended to dilute the supernatant with extract solution.

#### **Experimental examples:**

1. Take 0.1 g of enoki mushroom and add 1 mL of Extract solution for sample processing. The supernatant was diluted 2 times, and then proceeded according to the measurement procedure. Calculate  $\Delta A = A_T$  $A_{C}=1.454-0.047=1.407$ ,  $\Delta A_{S}=A_{S}-A_{B}=0.292-0.047=0.245$ . The enzyme activity is calculated according to the sample mass.

C1 Activity (U/g weight) =  $312.5 \times \Delta A \div \Delta A_S \div W \times 2$  (dilution times)=35893 U/g weight.

#### **Related products:**

β- 1,3-glucanase(β- 1,3-GA) Activity Assay Kit AK0556/AK0555

Cellulase(CL) Activity Assay Kit AK0211/AK0210

N-Acetyl-β-D-Glucosidase(NAG) Activity Assay Kit AK0088/AK0087

Hemicellulose Content Assay Kit AK0061/AK0060