

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

Operation Equipment: Spectrophotometer

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

Catalog Number: BC4300

Size: 50T/24S

Components:

Extract solution: 30mL \times 1. Storage at 4°C.

Reagent I: Powder \times 2. Storage at 4°C. Add 7 mL of distilled water to fully dissolve when the solution will be used. [Store unused reagents at 4°C for 2 weeks.](#)

Reagent II: 60 mL \times 1. Storage at 4°C.

Standard solution: 1mL \times 1, 5 μ mol/mL p-nitrophenol solution. The standard is diluted 20 times with reagent III to obtain a 0.25 μ mol/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, centrifuge, water-bath, transferpettor, 1 mL glass cuvette, 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 (10^4): 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 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	400			
Distilled water	-	400	400	500
Standard solution	-	-	100	-
sample	100	100	-	-
Reacting for 1 h at 37°C in a water bath.			-	-
Reagent II	1000	1000	1000	1000

Mix well, react for 2 minutes at RT. 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 μmol of p-nitrophenol every mg of protein in the reaction system per hour.

$$\text{C1 Activity (U/mg prot)} = \Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_S \div (C_{pr} \times V_S) \div T = 250 \times \Delta A \div \Delta A_S \div C_{pr}$$

2) Tissue weight

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

$$\text{C1 Activity (U/g weight)} = \Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_S \div (V_S \div V_E \times W) \div T = 250 \times \Delta A \div \Delta A_S \div W$$

3) Liquid

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

$$\text{C1 Activity (U/mL)} = \Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_S \div V_S \div T = 250 \times \Delta A \div \Delta A_S$$

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 μmol of p-nitrophenol every 10^4 cells or bacteria in the reaction system per hour at.

$$\text{C1 Activity (U/10}^4 \text{ cell)} = \Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_S \div (V_S \div V_E \times \text{cell amount}) \div T = 250 \times \Delta A \div \Delta A_S \div \text{cell amount};$$

V_S : Sample volume, 0.1mL

C_S : Standard concentration, 0.25 $\mu\text{mol/mL}$

V_E : Extract solution volume, 1 mL;

C_{pr} : Supernatant sample protein concentration (mg/mL);

T : Reaction time (min), 1 hour;

W : Sample weight, g;

Cell amount: 10 thousand as unit.

Note

1. If the absorbance value is greater than 1, 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 5 times, and then proceeded according to the measurement procedure. Calculate $\Delta A = A_T - A_C = 0.531 - 0.006 = 0.525$, $\Delta A_S = A_S - A_B = 0.305$. The enzyme activity is calculated according to the sample mass.

C1 Activity (U/g weight) = $250 \times \Delta A \div \Delta A_S \div W \times 5$ (dilution times) = 21516.4 U/g weight.

Related products:

BC0360/BC0365 β -1,3-glucanase(β -1,3-GA) Activity Assay Kit

BC2540/BC2545 Cellulase(CL) Activity Assay Kit

BC4290/BC4295 N-Acetyl- β -D-Glucosidase(NAG) Activity Assay Kit

BC4440/BC4445 Hemicellulose Content Assay Kit