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

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/ Microplate reader

Cat No: BC4305

Size: 100T/48S

Components:

Extract solution: Liquid 60mL×1, store at 4℃.

Reagent I: Powder×2, store at 4℃. Add 3 mL distilled water to each bottle before use, fully dissolved. The reagent should be prepared just before use and could be stored at 4℃ for 4 weeks.

Reagent II: Liquid 30mL×1, store at 4℃.

Standard solution: Liquid 1mL×1, 5μmol/mL p-nitrophenol solution, store at 4℃.

Product Description

β-1,4-glucanase/ cellobiohydrolase (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, balance, desk centrifuge, water bath/constant temperature foster box, ultrasonic cell disruptor, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ice and distilled water.

Procedure

I. Preparation of standard samples:

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℃ for 10min. Supernatant is placed on ice for test.

2. Bacteria or cells:

According to the number of cells (104): 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℃ for 10min. Supernatant is placed on ice for test.

3. Serum/plasma: direct measurement.

II. Determination

1. Preheat spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to

400 nm, set spectrophotometer counter to zero with distilled water.

2. Standard working solution: dilute the standard 16 times with distilled water to obtain 0.3125 μmol/mL standard solution.

3. 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℃.			-	-
Reagent II	200	200	200	200

Mix well, react for 2 minutes at RT. Take 200μL 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 $\Delta A = A_T - A_C$, $\Delta A_S = A_S - A_B$. Each test tube should be provided with one contrast tube. Standard curve and blank tube only need to be measured once or twice.

III. Calculate:

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.

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

C1 Activity ($U/10^4$ cell) =$\Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_S \div (V_S \div V_E \times$ cell amount$) \div T = 312.5 \times \Delta A \div \Delta A_S \div$cell amount

V_S: 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;
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) $=312.5\times\Delta A\div\Delta A_S\div W\times2$(dilution times)=35893 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

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