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: NA0309

Size: 100T/48S

Components:

Extract solution:60mL×1. Storage at 4℃.

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

Reagent II:30mL×1. Storage at 4℃.

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℃ 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℃ 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℃ in a water bath.			-	-
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 $\Delta A = A_T - A_C$, $\Delta A_S = A_S - A_B$.

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.

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

Related products：

NA0838/NA0596 β-1,3-glucanase(β-1,3-GA) Activity Assay Kit

NA0688/NA0447 Cellulase(CL) Activity Assay Kit

NA0312/NA0311 N-Acetyl-β-D-Glucosidase(NAG) Activity Assay Kit

NA0284/NA0283 Hemicellulose Content Assay Kit