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# 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:** AK0086

**Size:** 50T/24S

**Components:**

Extract solution: 30mL×1. Storage at 4℃ .

Reagent I: Powder×2. Storage at 4℃ . 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×1. Storage at 4℃ .

Standard solution: 1mL×1, 5μmol/mL p-nitrophenol solution. The standard is diluted 20 times with reagent III to obtain a 0.25μmol/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℃ 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 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℃ 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 ofp-nitrophenol every mg of protein in the reaction system per hour.

C1 Activity (U/mg prot)= $\Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_S \div (Cpr \times V_S) \div T = 250 \times \Delta A \div \Delta A_S \div Cpr$

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) = $\Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_S \div (V_S \div V_E \times W) \div T = 250 \times \Delta A \div \Delta A_S \div W$

3) Liquid

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

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

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 ofp-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$) \div T = 250 \times \Delta A \div \Delta A_S \div$cell amount;

$V_S$: Sample volume, 0. 1mL

Cs: Standard concentration, 0.25μmol/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, 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：**

| | |
|---|---|
| AK0556/AK0555 | β- 1,3-glucanase(β- 1,3-GA) Activity Assay Kit |
| AK0211/AK0210 | Cellulase(CL) Activity Assay Kit |
| AK0088/AK0087 | N-Acetyl-β-D-Glucosidase(NAG) Activity Assay Kit |
| AK0061/AK0060 | Hemicellulose Content Assay Kit |