

## **$\beta$ -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 $\times$ 1. Storage at 4°C .

Reagent I: Powder $\times$ 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 $\times$ 1. Storage at 4°C .

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

### **Product Description**

$\beta$ - 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  $\beta$ -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 ( $10^4$ ): 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/ 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( $\mu\text{L}$ )	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 $\mu\text{L}$  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  $\mu\text{mol}$  of p-nitrophenol every mg of protein in the reaction system per hour.

$$\text{C1 Activity (U/mg prot)} = \Delta A \div (\Delta A_S \div C_S) \times 1000 \times V_S \div (C_{pr} \times V_S) \div T = 312.5 \times \Delta A \div \Delta A_S \div C_{pr}$$

#### 2) Tissue weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1  $\mu\text{mol}$  of p-nitrophenol every gram of tissue in the reaction system per hour.

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

#### 3) Liquid

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

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

#### 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  $\mu\text{mol}$  of p-nitrophenol every  $10^4$  cells or bacteria in the reaction system per hour at.

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

$V_S$ : Sample volume, 0.02mL

$C_S$ : Standard concentration, 0.3125 $\mu\text{mol/mL}$

$V_E$ : 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 1.5, 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:

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