

Cellulase (CL) Assay Kit

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

Operation Equipment: Spectrophotometer/ microplate reader

Catalog Number: BC2545

Size:100T/48S

Components:

Extract reagent: 100mL×1, storage at 4°C.

Reagent 1: 5mL×1, storage at 4°C.

Reagent 2: 20mL×1, storage at 4°C.

Reagent 3: 5mL×1, storage at 4°C.

Standard: powder ×1, storage at 4°C. 10mg of anhydrous glucose (Loss on drying<0.2%), add 1mL of distilled water to dissolve before use, prepare a 10mg / mL glucose solution for future use, and store at 4 °C for 1 wee.

Prepared standard: The 10mg/mL standard solution dilute to 1, 0.8, 0.6, 0.4, 0.2, 0.1, 0 mg/mL for use.

Product Description:

Cellulase (EC 3.2.1.4) exists in bacteria, fungi and animals, which can catalyze cellulose degradation. It is a type of enzyme preparation that can be widely used in the fields of medicine, food, cotton spinning, environmental protection and renewable resource utilization.

The 3,5-dinitrosalicylic acid method is used to determine the reducing sugar content of cellulose catalyzed by CL.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/ microplate reader, adjustable transferpeltor, balance, mortar/homogenizer, centrifuge, micro glass cuvette/ 96 well flat-bottom plate, ice and distilled water.

Sample preparation:

1. Plant and animal tissues: Weigh about 0.1 g of sample, add 1 mL of Extract reagent and fully grind. Centrifugate at 8000g and 4°C for 10 min, the supernatants as samples to be tested.
2. Bacteria or cells: Collect the bacteria or cells into a centrifuge tube, discard the supernatant after centrifugation; add 1 mL of Extract reagent for every 5 million bacteria or cells, and break the bacteria or cells with an ultrasonic ice bath (power 20%, ultrasonic 3 seconds, interval 10 seconds, repeat 30 times); Centrifugate at 8000g and 4°C for 10 min, take the supernatant and place on ice for testing.

Procedure:

1. Preheat spectrophotometer/ microplate reader for 30min, adjust the wavelength to 540 nm and set the counter to zero with distilled water.
2. Add reagent to a 1.5mL EP tube:

| Reagent name (μL) | Control tube (Ac) | Test tube (At) | Standard tube (As) |
|-------------------|-------------------|----------------|--------------------|
| Reagent 1 | 50 | 50 | - |

| | | | |
|---|-----|-----|-----|
| Reagent 2 | 200 | 200 | - |
| Distilled water | 50 | 50 | - |
| Sample | | 50 | - |
| Boiled sample | 50 | | - |
| Mix well, and react accurately in water bath at 40°C for 30min. after taking out, put it in boiling water and boil for 15 min immediately to get the saccharification solution. | | | |
| Saccharification solution | 15 | 15 | - |
| Standard solution | - | - | 15 |
| Reagent 3 | 35 | 35 | 35 |
| Mix well, boil for 15min in a boiling water bath and cool. | | | |
| Distilled water | 250 | 250 | 250 |

Mix well, set the counter to zero with distilled water, and measure the absorbance A at 540 nm, and calculate $\Delta A = A_T - A_C$.

Calculation:

1. set the counter to zero with the standard tube 0mg/mL at 540 nm and read the absorbance value a of the standard tube. According to concentration of standard solution and absorbance to create the standard curve, take standard solution as Y-axis, ΔA s as X-axis. Take ΔA into the equation to obtain y (mg/mL).

2. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 μ g glucose per minute in the reaction system every milligram tissue protein

$$CL (U/mg \text{ prot}) = 1000 \times y \times V_{rv} \div (V_s \times C_{pr}) \div T = 233y \div C_{pr}$$

3. Sample weight:

Unit definition: One unit of enzyme activity is defined as that one gram tissue catalyzes the production of 1 μ g glucose per min in the reaction system.

$$CL (U/g) = 1000 \times y \times V_{rv} \div (V_s \times W \div V_e) \div T = 233y \div W$$

4. Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as that 10⁴ cells or bacteria catalyzes the production of 1 μ g glucose in the reaction system per min.

$$CL (U/10^4 \text{ cell}) = 1000 \times y \times V_{rv} \div (500 \times V_s \div V_e) \div T = 0.467 \times y$$

$$1000: 1\text{mg/mL} = 1000\mu\text{g/mL}$$

V_{rv} : Total volume of reaction system, 0.35mL.

V_s : sample volume added, 0.05mL;

V_e : volume used in the extraction solution, 1mL;

C_{pr} : sample protein concentration, mg/mL;

W : Fresh weight of sample, g;

T : React time, 30min.

500: the number of cells or bacteria, 500 \times 10 thousand.

Recent Product Citations:

Guo Q, Du G, Qi H, et al. A nematicidal tannin from Punica granatum L. rind and its physiological effect on pine wood nematode (Bursaphelenchus xylophilus)[J]. Pesticide biochemistry and physiology,

2017, 135: 64-68.

References:

Faria M L, Kolling D, Camassola M, et al. Comparison of *Penicillium echinulatum* and *Trichoderma reesei* cellulases in relation to their activity against various cellulosic substrates[J]. *Biores. Technol*, 2008, 99: 1417-1424.

Related Products:

BC0340/BC0345 Glucogen Content Assay Kit
BC2450/BC2455 Plant Tissue Fructose Content Assay Kit
BC2510/BC2515 Trehalase Activity Assay Kit