# Cellulase (CL) Activity 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:** 60mL×1, storage at 2-8℃.

**Reagent I:** 5mL×1, storage at 2-8℃.

**Reagent II:** 20mL×1, storage at 2-8℃.

**Reagent III:** 5mL×1, storage at 2-8℃.

**Standard:** powder ×1, storage at 2-8℃. Containing 10 mg of anhydrous glucose (loss on drying < 0.2%). Add 1 mL of distilled water to dissolve it for standby before use. It can be stored for two week at 4℃, or it can be stored for a longer time with saturated benzoic acid solution.

**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, water bath, transferpettor, 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℃ for 10 min, take the supernatant and place on ice for testing.
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 200w, ultrasonic 3 seconds, interval 10 seconds, repeat 30 times); Centrifugate at 8000g and 4℃ 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 spectrophotometer set the counter to zero with distilled water.
2. Standard preparation: Dilute the standard with distilled water to 1, 0.8, 0.6, 0.4, 0.2, 0.1, 0 mg/mL.



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3. Add reagent to a 1.5mL EP tube:

| Reagent name（μL） | Control tube (Ac) | Test tube (At) | Standard tube (As) |
|---|---|---|---|
| Reagent I | 50 | 50 | - |
| Reagent II | 200 | 200 | - |
| Distilled water | 50 | 50 | - |
| Sample | | 50 | - |
| Boiled sample | 50 | | - |
| Mix well, and react accurately in water bath at 40℃ 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 III | 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 $\Delta A = A_T - A_C$. $\Delta As$ = As-A(0 mg/mL). Standard curves only need to be done 1-2 times.

**Calculation:**

1. Standard curve

A standard curve was created from the absorbance (x, ΔAs) and concentration (y, mg/mL) of the standard tube, and ΔAt (x, ΔAt) was brought into the standard curve to calculate the amount of product y (mg/mL) generated by the sample.

2. Calculation

(1)Protein concentration:

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

CL (U/mg prot) = 1000 ×y ×Vrv÷ （Vs ×Cpr） ÷T = 233y÷Cpr

(2)Sample weight:

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

CL (U/g) =1000× y×Vrv÷ （Vs×W÷Ve） ÷T = 233y÷W

(3)Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as that $10^4$ cells or bacteria catalyzes the production of 1μg glucose in the reaction system per min.

CL (U/$10^4$ cell) = 1000× y×Vrv÷(500×Vs÷Ve) ÷T=0.467×y

1000: 1mg/mL = 1000μg/mL

Vrv: Total volume of reaction system, 0.35mL.

Vs: sample volume added, 0.05mL;

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

Cpr: sample protein concentration, mg/mL;

W: Fresh weight of sample, g;

T: React time, 30min.

500: the number of cells or bacteria, 5 million

**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 Pennicillium echinulatum and Trichoderma reesei cellulases in relation to their activity against various cellulosic substrates[J]. Biores. Technol, 2008, 99: 1417-1424.

**Related Products:**

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