



## Cellulase Microplate Assay Kit

### Product Information

Cat.No. Kit-3432

### Product Overview

The enzyme catalysed reaction products can be measured at a colorimetric readout at 540 nm.

### Description

Cellulases are a family enzymes that include  $\beta$ -Glucosidases, endoglucanases, and exoglucanases. These enzymes cleave the  $\beta$ -1,4-D-glycosidic bonds that link the glucose units comprising cellulose. In addition to being produced by plants, cellulase activity is found in many fungi and bacteria, including some plant pathogens. Most animal cells are not known to produce cellulase; cellulolytic activity is often carried out in animals by symbionts. However, recent evidence does suggest cellulase production in some animals, such as insects and arthropods. The study of cellulase activity has many applications in plant molecular biology, agriculture, and manufacturing. Cellulase is also becoming important in the development of alternative fuel sources, as glucose obtained from cellulose hydrolysis is easily fermented into ethanol.

### Storage

Shipped and store at 4 degree C for 6 months.

### Size

100 Assays

### Kit Components

96-Well Microplate: 1 plate  
Assay Buffer: 30 ml x 4, 4 °C  
Substrate: Powder x 1, 4 °C  
Reaction Buffer: 5 ml x 1, 4 °C  
Dye Reagent: 10 ml x 1, 4 °C  
Standard (500  $\mu$ g/ml): 1ml x 1, 4 °C  
Plate Adhesive Strips: 3 Strips

### Materials Required but Not Supplied



## Cellulase Microplate Assay Kit

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice
9. Convection oven

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### Preparation

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonication 3s, interval 10s, repeat 30 times); centrifuged at 10,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

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### Assay Protocol

Add following reagents in the microplate:

Reagent Sample Control Standard Blank

Sample 10 µl -- -- --

Assay Buffer -- 10 µl -- --

Reaction Buffer 50 µl 50 µl -- --

Substrate 40 µl 40 µl -- --

Mix, put it in the oven, 37 °C for 30 minutes.

Standard -- -- 100 µl

Distilled water -- -- -- 100 µl

Dye Reagent 100 µl 100 µl 100 µl 100 µl



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Mix, put it into the convection oven, 90 °C for 10 minutes, record absorbance measured at 540nm.

### Analysis

Unit Definition: One unit of Cellulase activity is the enzyme that generates 1 µg of reducing sugar per minute.

1. According to the protein concentration of sample

$$CL (U/mg) = \frac{C_{Standard} \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Standard} / (C_{Protein} \times V_{Sample})} / T = \frac{166.7 \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) / C_{Protein}}$$

2. According to the weight of sample

$$CL (U/g) = \frac{C_{Standard} \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Standard} / (V_{Sample} \times W / V_{Assay})} / T = \frac{166.7 \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) / W}$$

3. According to the quantity of cells or bacteria

$$CL (U/10^4) = \frac{C_{Standard} \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Standard} / (V_{Sample} \times N / V_{Assay})} / T = \frac{166.7 \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) / N}$$

C<sub>Protein</sub>: the protein concentration, mg/ml;

C<sub>Standard</sub>: the concentration of Standard, 500 µg/ml;

W: the weight of sample, g;

V<sub>Total</sub>: the total volume of the enzymatic reaction, 0.1 ml;

V<sub>Standard</sub>: the volume of standard, 0.1 ml;

V<sub>Sample</sub>: the volume of sample, 0.01 ml;

V<sub>Assay</sub>: the volume of Assay buffer, 1 ml;

T: the reaction time, 30 minutes;

N: the quantity of cell or bacteria, N × 10<sup>4</sup>

### Sensitivity

50 µg/mL - 500 µg/mL