

Cellulase Microplate Assay Kit

Basic information:

Catalog No.: UAK1095

Size: 100 Assays

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Cellulases are a family of enzymes that include β -Glucosidases, endoglucanases, and exoglucanases. These enzymes cleave the β -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.

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

II. KIT COMPONENTS

Component	Volume	Storage
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 μ g/ml)	1ml x 1	4 °C
Plate Adhesive Strips	3 Strips	

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Note:

Substrate: add 5 ml distilled water to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

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

IV. SAMPLE 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 5×10^6 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.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

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

VI. CALCULATION

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

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

$$= 166.7 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 166.7 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

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

$$= 166.7 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the concentration of Standard, 500 µg/ml;

W: the weight of sample, g;

V_{Total} : the total volume of the enzymatic reaction, 0.1 ml;

V_{Standard} : the volume of standard, 0.1 ml;

V_{Sample} : the volume of sample, 0.01 ml;

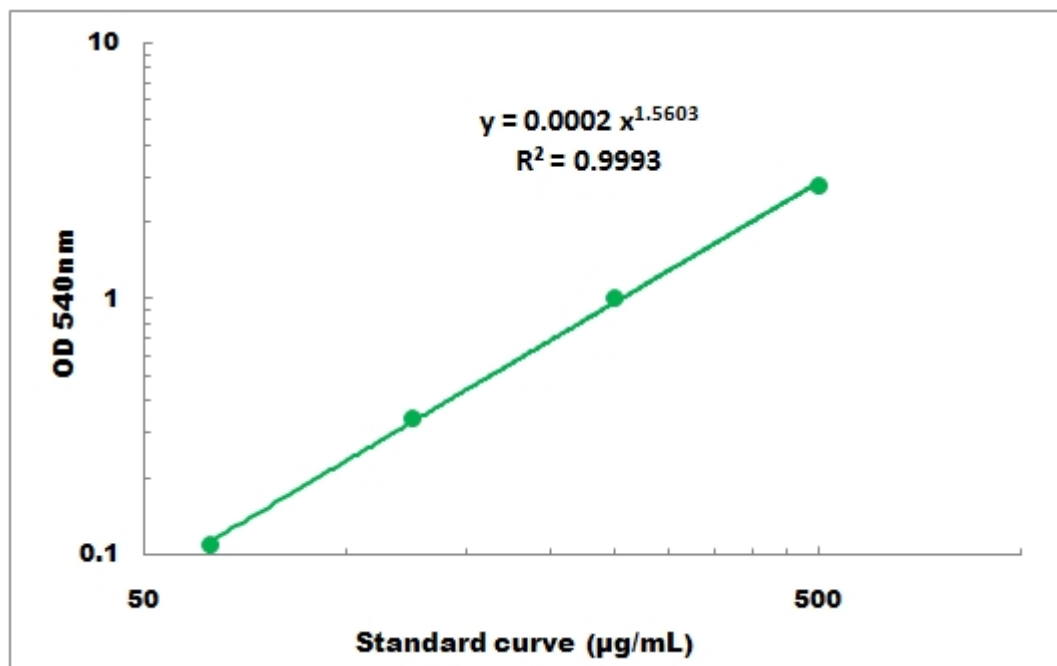
V_{Assay} : the volume of Assay buffer, 1 ml;

T: the reaction time, 30 minutes;

N: the quantity of cell or bacteria, $N \times 10^4$.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 50 µg/mL - 500 µg/mL