CREATIVE BIOMART®
Assay Kit

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 ß-Glucosidases, endoglucanases, and exoglucanases. These enzymes cleave the ß-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 arthopods. 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 µg/ml): 1ml x 1, 4 °C
Plate Adhesive Strips: 3 Strips

Materials Required but Not Supplied

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.org
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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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

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, intervation 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.

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) = CStandard × (ODSample - ODControl) / (ODStandard - ODBlank) × VStandard / (CProtein × VSample) / T = 166.7 × (ODSample - ODControl) / (ODStandard - ODBlank) / CProtein

2. According to the weight of sample

CL (U/g) = CStandard × (ODSample - ODControl) / (ODStandard - ODBlank) × VStandard / (VSample × W / VAssay) / T = 166.7 × (ODSample - ODControl) / (ODStandard - ODBlank) / W

3. According to the quantity of cells or bacteria

CL (U/10^4) = CStandard × (ODSample - ODControl) / (ODStandard - ODBlank) × VStandard / (VSample × N/ VAssay) / T = 166.7 × (ODSample - ODControl) / (ODStandard - ODBlank) / N

CProtein: the protein concentration, mg/ml;

CStandard: the concentration of Standard, 500 μg/ml;

W: the weight of sample, g;

VTotal: the total volume of the enzymatic reaction, 0.1 ml;

VStandard: the volume of standard, 0.1 ml;

VSample: the volume of sample, 0.01 ml;

VAssay: the volume of Assay buffer, 1 ml;

T: the reaction time, 30 minutes;

N: the quantity of cell or bacteria, N × 10^4

Sensitivity

50 μg/mL - 500 μg/mL

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