Cellulose (CLL) Content Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC4285

Size:100T/96S

Components:

Extract solution I: 400 mL of 80% ethanol; 320 mL of anhydrous ethanol and 80 mL of distilled water are mixed for self-preparation.

Extract solution II: 100 mL×1. Storage at 4℃ and protect from light.

Reagent I: Powder×2. Storage at 4℃ and protect from light.

Reagent II: 10 mL×1. Storage at 4℃ and protect from light.

Standard: powder×1, containing 10 mg of anhydrous glucose. Add 1 mL of distilled water to dissolve it for standby before use to prepare 10 mg/mL anhydrous glucose. Reagents can be stored for two weeks at 4°C

Preparation of working solution: Take a bottle of Reagent I and add 2.5 mL of Reagent II, mix well, if it is difficult to dissolve, it can be fully shaken or heated and stirred; the unused reagent can be stored at 4°C for a week.

Product Description

Cellulose (CLL) is a straight chain polymer composed of β-D-glucose units linked by β-1,4-glycoside bond, which is usually combined with hemicellulose, pectin and lignin, and is the main structural component of plant cell wall. Cellulose products are widely used in food, paper, plastics, explosives, electrical engineering and scientific research equipment.

Cellulose can be decomposed into β-D-glucose by heating in acid condition. Under the condition of strong acid, the content of cellulose is determined with anthrone chromogenic agent.

Reagents and Equipment Required but Not Provided.

Spectrophotometer/microplate reader, low temperature centrifuge, water-bath, micro glass cuvette/96 well flat-bottom plate, ransferpettor, mortar/homogenizer, ice, acetone, concentrated H_2SO_4, anhydrous ethanol, distilled water and EP tube.

Procedure

I. Extraction of Cellulose

a. Bacteria or cell treatment:

Extraction of cell wall substance (CWM): Weigh about 0.3 g (recorded as W1) of sample, add 1 mL of Extract solution I, homogenize rapidly at room temperature, water bath with 90℃ for 20 minutes, cool to room temperature. Centrifugate at 6000 ×g for 10 minutes at 25℃, discard the supernatant. The

precipitate is washed twice with 1.5 mL of Extract solution I and acetone respectively (vortex shaking for 2 minutes, centrifugate at 6000 ×g for 10 minutes at 25℃, and then the supernatant is discarded). The precipitate is the rough cell wall. Add 1 mL of Extract solution II (starch removal) and soak for 15 hours, centrifugate at 6000 ×g for 10 minutes at 25℃, then discard the supernatant. Dry the precipitate to obtain the cell wall substance (CWM), and weigh it as W2.

b. Tissue:

Weigh about 5 mg of dried CWM (record as W3), add 0.5 mL of distilled water to fully homogenate, transfer the homogenate to EP tube, fix the volume to 0.5 mL with distilled water. Put it into ice-water mixture, slowly add 0.75 mL of concentrated H_2SO_4, slowly mix, and let it stand in ice-water bath for 30 minutes. Centrifuged at 8000 ×g for 10 minutes at 4℃, the supernatant is taken, diluted with distilled water for 20 times, and then to be test.

Note: In the process of extracting cellulose, first of all, the EP tube placed in the ice-water bath needs to be fixed, not floating up, down, left or right. On the one hand to ensure your own safety, on the other hand to prevent the ice-water mixture from entering the EP tube and causing test error. Furthermore, when adding concentrated H_2SO_4, it is recommended that the pipette tip be extended below the sample liquid level, and add slowly to prevent the liquid level from boiling and sample carbonization.

II. Measurement steps

a. Preheat the spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 620 nm and the spectrophotometer adjust zero with distilled water.

b. Dilute 10 mg/mL standard solution with distilled water to 0.125, 0.0625, 0.03125, 0.015625, 0.0078, 0.0039 and 0.00195 mg/mL standard solution for standby.

c. Add the following reagents successively into the 1.5 mL EP tube:

Reagent name (μL)	Test tube (T)	Standard tube (S)	Blank tube (B)
Sample	150	-	-
Standard solution	-	150	-
Distilled water	-	-	150
Working solution	35	35	35
Concentrated H_2SO_4	315	315	315
Mix well, put it in a 95℃-water bath for 10 minutes (cover tightly to prevent water loss), take it out and cool it to room temperature. Take 200 μL and measure the absorption value at 620 nm in micro glass cuvette/96 well plate, and record it as A_T, A_S and A_B respectively. Calculate $\Delta A = A_T - A_B$, $\Delta A_S = A_S - A_B$. Blank tube and standard curve only need to be tested 1-2 times.			

III. Calculation of reducing sugar content:

1. Drawing of standard curve:

According to the concentration of the standard tube (x, mg/mL) and the absorbance ΔA standard (y, ΔA standard), establish a standard curve. According to the standard curve, bring ΔA (y, ΔA) into the formula to calculate the sample concentration (x, mg/mL).

2. Calculation of cellulose content:

(1) Calculate by the quality of sample:

Cellulose (mg/g mass) =x×V_{EV}×20×(W2÷W3)÷W1÷1.11=22.52x×W2÷W3÷W1

(2) Calculate by sample cell wall mass (CWM):

Cellulose (mg/g dry weight) =x×V_{EV}×20÷W3÷1.11=22.52x÷W3

1.11: The constant of converting glucose content measured by this method into cellulose content, i.e. 111 μg of glucose is colored by anthrone reagent equivalent to the color displayed by 100 μg of cellulose anthrone reagent;

V_{EV}: The volume of cellulose extract solution, 1.25 mL (0.5 mL of distilled water+0.75 mL of concentrated H_2SO_4);

20: The dilution ratio of sample;

W1: Sample mass, 0.3 g;

W2: Weight of sample cell wall substance (CWM) quality, g;

W3: Weight of cell wall substance (CWM) weighed when extracting cellulose, 0.005 g.

Note:

1. If the measured absorbance value exceeds the absorbance value in the linear range, you can increase the sample volume or dilute the sample before performing the measurement.
2. Concentrated H_2SO_4 is highly corrosive. Special attention shall be paid to each step during operation: when adding concentrated H_2SO_4 to cellulose extraction, it is recommended that the pipette tip be extended below the sample liquid level and added slowly to prevent liquid splashing and burning; after taking out in 95℃ water bath, the EP tube cover shall be cooled to room temperature and then opened to prevent liquid splashing and burning.
3. Due to the strong volatility and pungent smell of Reagent II, it is recommended to prepare the working solution in the fume hood.

Experimental examples:

1. Take 0.3 g of carnation leaves to extract the cell wall material, and the mass of the cell wall material (CWM) is 0.02 g. Weigh about 5mg of dried CWM (denoted as W3) to extract cellulose. Dilute the supernatant 20 times with distilled water and follow the measurement procedure. After determination with 96 well flat-bottom plate, calculate $\Delta A=A_T-A_B$=0.538-0.061=0.477. Bring the result into the standard curve y=8.3536x+0.0388, and calculate x=0.05246. The content is calculated according to the sample mass.
   Cellulose（mg/g mass）=22.52x×W2÷W3÷W1=15.752 mg/g mass。
2. Take 0.3 g of ryegrass to extract the cell wall material, and the mass of the cell wall material (CWM) is 0.04 g. Weigh about 5mg of dried CWM (denoted as W3) to extract cellulose. Dilute

the supernatant 20 times with distilled water and follow the measurement procedure. After determination with 96 well flat-bottom plate, calculate $\Delta A=A_T-A_B=0.666-0.061=0.605$. Bring the result into the standard curve

y=8.3536x+0.0388, and calculate x=0.06778. The content is calculated according to the sample mass.

Cellulose（mg/g mass）=22.52x×W2÷W3÷W1=40.704 mg/g mass。

Related products:

BC3330/BC3335	Glycogen synthase(GCS) Activity Assay Kit
BC3360/BC3365	UDP-glucose pyrophosphosphprylase(UGP) Activity Assay Kit
BC4440/BC4445	Hemicellulose Content Assay Kit

Technical Specifications:

Minimum Detection Limit：0.0037 mg/mL

Linear Range：0.00391-0.3 mg/mL