Lignin Assay Content Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC4205

Size: 100T/96S

Components:

Reagent I: Liquid 35 mL×1. Storage at 2-8℃.

Reagent II: Liquid 35 mL×1. Storage at 2-8℃.

Reagent III: Self-provided Petroleum ether, about 35 mL, storage at 2-8℃. (boiling range : 30 - 60 °C)

Reagent IV: Liquid 34 mL×1. Storage at 2-8℃. The reagent has strong volatility and certain toxicity, pay attention to the operation in the fume cupboard and seal with sealing film after use;

Reagent V: Liquid 1.5 mL×1. Storage at 2-8℃.

Reagent VI: Liquid 35 mL×1. Storage at 2-8℃. The reagent is corrosive, pay attention to protection

Product Description

Lignin is one of the components of plant cell wall. It has the function of connecting cells. Lignin exists in xylem. The main function is to harden cell wall by forming interwoven net, which is the main component of secondary wall.

There is a characteristic absorption peak at 280 nm after acetylation of phenolic hydroxyl in lignin. The absorbance value of 280 nm is positively correlated with lignin content.

Reagents and Equipment Required but Not Provided.

Ultraviolet spectrophotometer, table centrifuge, water-bath, 1mL quartz cuvette, transferpettor, mortar, EP tube, glacial acetic acid, Petroleum ether and distilled water.

Procedure

I. Crude enzyme extraction:

Dry the sample to constant weight at 80℃, crush it, pass 40 mesh sieve, weigh about 3 mg into 1.5 mL EP tube.

II. Determination Procedure

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 280 nm and set the counter to zero with glacial acetic acid.

2. Operation table: (in 1.5 mL centrifuge tube)

(1) Acetylization

	Test tube (A_T)	Blank tube (A_B)
Sample (mg)	3	-
Reagent I（μL）	300	-
65 °C reaction for 30 min. 8000g, centrifuge at room temperature for 5min; remove supernatant and leave sediment.		
Reagent II（μL）	300	-
Vortex oscillation for 5min。8000g, centrifuge at room temperature for 5min; remove supernatant and leave sediment.		
Reagent III (μL）	300	-
Vortex oscillation for 5min。8000g, centrifuge at room temperature for 5min; remove supernatant and leave sediment.		
Reagent IV（μL）	300	300
Reagent V（μL）	12	12
Seal with sealing film. Mix thoroughly. Acetylated in 80℃-water bath for 40 min. Shake every 10 minutes. Then cool naturally.		
Reagent VI（μL）	300	300
Mix thoroughly. Centrifugate at room temperature, 8000 g for 10 min. Take the supernatant for test.		

(2) Absorbance measured: (**Because glacial acetic acid is volatile, please determination immediately after mix! Suggest mixing a tube to test a tube**)

Supernatant	12	12
Glacial acetic acid	588（Reference "**Note**"）	588（Reference "**Note**"）
Mix thoroughly. Take out 200 μL into micro quartz cuvette/96 well UV plate to measure the absorption value A at 280 nm. Record as A_T, A_B. $\Delta A=A_T-A_B$.		

III. Calculation of lignin:

a. Micro quartz cuvette

Lignin content (mg/g) $=\Delta A\div\varepsilon\div d\times V_T\div(V_S\times W\div V_A)=1.3105\times\Delta A\div W$

Percentage content of lignin (%) = lignin content÷1000×100%

V_A: Volume of acetylation reaction, 1.02mL;

ε: Extinction coefficient of lignin, 23.35 mL/mg/cm;

d: Light diameter of cuvette, 1 cm;

V_S: Volume of supernatant, 0.012 mL;

V_T: Detection volume,1 mL;

W: Sample weight, g;

1000: Conversion factor, 1 g=1000 mg.

Note:

1. Reagent IV is toxic. Please take protective measures during operation. Sealing film must be used before heating to prevent gas overflow.
2. There is violent reaction during heating. Shake gently when shaking to avoid personal injury caused by excessive pressure.
3. Glacial acetic acid has strong irritation. It is recommended that the operation process be operated in the fume hood.
4. Take the supernatant and add glacial acetic acid according to the degree of acetylation of the sample. The amount of glacial acetic acid can be adjusted. Ensure that the absorption value is between 0.1-0.8. And participate in the calculation in the formula.
5. Because glacial acetic acid is volatile. It is suggested to use a cuvette for color experiment.

Related publications:

[1] Liang R, Zhao J, Li B, et al. Implantable and degradable antioxidant poly (ε-caprolactone)-lignin nanofiber membrane for effective osteoarthritis treatment[J]. Biomaterials, 2020, 230: 119601.

References:

[1] Goldschmid O. Determination of phenolic hydroxyl content of lignin preparations by ultraviolet spectrophotometry[J]. Analytical Chemistry, 1954, 26(9): 1421-1423.

[2] Janshekar H, Brown C, Fiechter A. Determination of biodegraded lignin by ultraviolet spectrophotometry[J]. Analytica Chimica Acta, 1981, 130(1): 81-91.

Related Products:

BC2030/BC2035 Isocitrate Lyase (ICL) Activity Assay Kit

BC3170/BC3175 Acetokinase (ACK) Activity Assay Kit