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Lignin Assay Content Kit

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

Operation Equipment: Spectrophotometer/ microplate reader

Catalog Number: AK0103

Size: 100T/96S

Components:

Reagent I: Liquid 34 mL×1. Storage at 4℃ . Seal with sealing film after use;

Reagent Ⅱ: Liquid 35 mL×1. Storage at 4℃ .

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/microplate reader, table centrifuge, water-bath, micro quartz cuvette/96 well UV flat-bottom plate (non-polystyrene material), transferpettor, mortar/ homogenizer, EP tube, parafilm, perchloric acid, glacial acetic acid 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/96 well flat bottom plate)

Reagent Name (μL)	Test tube (A_T)	Blank tube (A_B)
Sample (mg)	3	-
Reagent I	300	300
Perchloric acid	12	12
Seal with sealing film. Mix thoroughly. Acetylated in 80℃-water bath for 40 min. Shake every 10 minutes. Then cool naturally.		
Reagent II	300	300
Mix thoroughly. Centrifugate at room temperature, 8000 g for 10 min. Take the supernatant for test.		

Supernatant	12	12
Glacial acetic acid	588	588
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%=0. 13105×ΔA÷W

V_A: Volume of acetylation reaction, 0.612 mL;

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,0.6 mL;

W: Sample weight, g;

1000: Conversion factor, 1 g=1000 mg.

b. 96 well flat-bottom plate

The optical diameter d=1 cm of the cuvette in the above formula is changed to 0.6 cm of the 96 well UV plate.

Note:

1. Reagent I 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:

AK0246/AK0245 Isocitrate Lyase (ICL) Activity Assay Kit

AK0149/AK0148 Acetokinase (ACK) Activity Assay Kit