Lignin Content Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: NA0333

Size: 50T/48S

Components:

Reagent I: Liquid 30 mL×1. Storage at 4℃. Seal with sealing film after use;

Reagent II: Liquid 30 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.

Spectrophotometer, table centrifuge, water-bath, 1 mL quartz cuvette, 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 5 mg into 1.5 mL EP tube.

II. Determination Procedure

1. Preheat the ultraviolet 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)

Reagent Name (μL)	Test tube (A_T)	Blank tube (A_B)
Sample (mg)	5	-
Reagent I	500	500
Perchloric acid	20	20
Seal with sealing film. Mix thoroughly. Acetylated in 80℃ water bath for 40 min. Shake every 10 minutes. Then cool naturally.		
Reagent II	500	500
Mix thoroughly. Centrifugate at room temperature, 8000 g for 10 min. Take the supernatant for test.		
Supernatant	20	20

Glacial acetic acid	980	980
Mix thoroughly. Measure the absorption value A at 280 nm. Record as A_T, A_B. $\Delta A=A_T-A_B$.		

III. Calculation of lignin:

Lignin content (mg/g) $=\Delta A\div\varepsilon\div d\times V_T\div(V_S\times W\div V_A)=2.184\times\Delta A\div W$

Percentage content of lignin (%) =lignin content÷1000×100%=$0.2184\times\Delta A\div W$

V_A: Volume of acetylation reaction, 1.02 mL;

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

d: Light diameter of cuvette, 1 cm;

V_S: Volume of supernatant, 0.02 mL;

V_T: Detection volume, 1 mL;

W: Sample weight, g;

1000: Conversion factor, 1 g=1000 mg.

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.

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:

NA0724/NA0482 Isocitrate Lyase (ICL) Activity Assay Kit

NA0640/NA0377 Acetokinase (ACK) Activity Assay Kit