Lignin Assay Kit (Microanalysis)

Description

Lignin is one of the major components of plant cell walls. It is a class of polymeric substances present in lignified tissues and primarily functions to strengthen plant structures by forming an interwoven network. Lignin is mainly located between fibers, where it contributes to compressive strength.

Detection Principle

Following acetylation of the phenolic hydroxyl groups in lignin, a characteristic absorption peak is generated at 280 nm. The absorbance at 280 nm is positively correlated with lignin content.

Specifications

Composition and Storage Conditions (100T/96S):

Component	Specification	Storage Condition
CB0280M-A	35 mL × 1	Store at 4°C
CB0280M-B	35 mL × 1	Store at 4°C
CB0280M-C	Self-provided	Store at 4°C; Approximately 35 mL petroleum ether required (boiling range: 30–60°C)
CB0280M-D	34 mL × 1	Store at 4°C
CB0280M-E	1.5 mL × 1	Store at 4°C
CB0280M-F	35 mL × 1	Store at 4°C

Note: Before formal measurement, it is recommended to select 2–3 samples with expected large differences for a preliminary experiment.

Instructions

I. Required Equipment and Materials:

UV spectrophotometer/microplate reader, benchtop centrifuge, water bath, quartz microcuvette/96-well UV plate (non-polystyrene material), adjustable pipettes, mortar, EP tubes, sealing film, glacial acetic acid, petroleum ether, 30–50 mesh sieve, and distilled water.

II. Sample Preparation:

Dry the sample at 80°C until a constant weight is reached, grind thoroughly, pass through a 30–50 mesh sieve, and weigh approximately 3 mg into a 1.5 mL EP tube.

III. Assay Procedure:

1. Instrument Preparation

Preheat the spectrophotometer/microplate reader for at least 30 min, set the wavelength to 280 nm, and zero the spectrophotometer with glacial acetic acid.

2. Assay Procedure

Acetylation:

Reagent Name	Sample Tube (μL)	Blank Tube (μL)
Sample (mg)	3	
CB0280M-A	300	
Incubate in a 65°C water bath for 30 min. Centrifuge at 8000 g for 5 min at room temperature. Discard the supernatant and retain the pellet.		
CB0280M-B	300	
Vortex for 5 min. Centrifuge at 8000 g for 5 min at room temperature. Discard the supernatant and retain the pellet.		
CB0280M-C	300	
Vortex for 5 min. Centrifuge at 8000 g for 5 min at room temperature. Discard the supernatant and retain the pellet.		
CB0280M-D	300	300
CB0280M-E	12	12
Mix thoroughly and seal with sealing film to prevent moisture loss. Incubate in an 80°C water bath for 40 min to perform acetylation. Gently shake for 30 s every 10 min during incubation, then allow the reaction mixture to cool naturally to room temperature.		
CB0280M-F	300	300
Mix thoroughly and centrifuge at 8000 g for 10 min at room temperature. Collect the supernatant for analysis.		

Measurement:

Reagent Name	Sample Tube (μL)	Blank Tube (μL)
Supernatant	12	12
Glacial acetic acid	588	588
Mix thoroughly and transfer 200 μL into a quartz microcuvette or a 96-well UV plate. Measure the absorbance at 280 nm and record as A_sample and A_blank, respectively. Calculate: $\Delta A = A\_sample - A\_blank$ Note: The Blank Tube only needs to be measured 1–2 times.		

IV. Calculation:

1. Calculation Using a Quartz Microcuvette

Lignin content (mg/g) = $\Delta A \div \varepsilon \div d \times V\_detection \div (V\_supernatant \times W \div V\_acetylation)$

$= 1.3105 \times \Delta A \div W$

Lignin percentage (%) = Lignin content ÷ 1000 × 100%

Note:

V_acetylation: Total acetylation reaction volume, 0.612 mL

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

d: Optical path length of the cuvette, 1 cm

V_supernatant: Volume of supernatant used, 0.012 mL

V_detection: Detection volume, 0.6 mL

W: Sample weight (g)
1000: Conversion factor (1 g = 1000 mg)

2. Calculation Using a 96-Well UV Plate

For calculations using a 96-well UV plate, replace d = 1 cm in the above formula with d = 0.5 cm (optical path length of a 96-well plate).

Precautions

1. **CB0280M-D is toxic.** Appropriate protective measures should be taken during operation. The reaction mixture must be sealed with sealing film before heating to prevent gas leakage.
2. A vigorous reaction occurs during heating. Gently shake the tubes during mixing to avoid excessive pressure buildup that may cause splashing and personal injury.
3. Glacial acetic acid is highly irritating. It is recommended that all procedures be performed in a fume hood.
4. The volume of glacial acetic acid added during the measurement step may be adjusted according to the degree of sample acetylation to ensure that the absorbance falls within the range of **0.1–0.8**. Any volume adjustment should be incorporated into the calculation.
5. Due to the volatility of glacial acetic acid, measurement using a cuvette is recommended. Absorbance should be measured immediately after mixing.
6. **CB0280M-E is corrosive.** Appropriate protective measures should be taken during handling.
7. This product is intended for professional scientific research use only. It must not be used for clinical diagnosis or treatment, food or drug applications, and must not be stored in residential environments.
8. For your safety and health, please wear a lab coat and disposable gloves during operation.