CheKine™ Mirco Lignin Content Assay Kit

Cat #: KTB3027 Size: 48 T/48 S 96 T/96 S

	Mirco Lignin Content Assay Kit		
REF	**Cat #:** KTB3027	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Lignin is one of the components that make up plant cell walls. It is a class of substances composed of polymerized aromatic alcohols and exists in lignified tissues, primarily serving to harden the cell walls by forming an interwoven network. Lignin is mainly located between cellulose fibers, providing compressive strength. CheKine™ Mirco Lignin Content Assay Kit offers a simple, convenient, and rapid method for detecting lignin content, suitable for plant tissue samples. The principle of the kit is based on the characteristic absorption peak at 280 nm after acetylation of the phenolic hydroxyl groups in lignin, with the absorbance value at 280 nm being positively correlated with lignin content.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	30 mL	60 mL	4℃, protected from light
Reagent Ⅱ	30 mL	60 mL	4℃
Reagent Ⅲ	60 mL	120 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 280 nm
- 96-well quartz plate (non-polystyrene material) or microquartz cuvette, precision pipettes, disposable pipette tips
- Oven, 40-mesh sieve, analytical balance, glass test tubes, sealing film, water bath
- Deionized water, perchloric acid, glacial acetic acid

Reagent Preparation

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃. If frozen, thaw in a 37℃ water bath immediately before use.

Note: Reagent I is toxic, and both Reagent I and Reagent III have a strong odor. It is recommended to perform the experiment in a fume hood.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

Plant Tissue: Dry the sample at 80℃ until constant weight (approximately 2 h), crush it, pass it through a 40-mesh sieve, and weigh approximately 2 mg into a 10 mL glass test tube (use a glass test tube, do not use an EP tube).

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 280 nm, ultraviolet spectrophotometer was returned to zero with glacial acetic acid.

2. Sample measurement. (The following operations are performed in a 10 mL glass test tube.)

Reagent	Test Tube (μL)	Blank Tube (μL)
Sample (mg)	2	0
Reagent I	500	500
Perchloric acid	20	20
In a 10 mL glass test tube, seal with sealing film, mix thoroughly, place in an 80℃ water bath for 40 min, shaking once every 10 min, then allow to cool naturally **Note: Perchloric acid is highly corrosive and irritating. Handle with care in a fume hood.**		
Reagent II	500	500
Mix thoroughly and let stand for 2 min		
Supernatant	20	20
Reagent III	980	980
Mix well, and immediately transfer 200 μL into a micro quartz cuvette or 96-well quartz plate (non-polystyrene material), measure the absorbance A at 280 nm, and record as A_{Test} and A_{Blank}, respectively. Calculate $\Delta A=A_{Test}-A_{Blank}$.		

Note:1. Reagent I is toxic. Please take proper precautions when handling it, and ensure it is sealed with sealing film before heating to prevent gas from escaping.

2. There is a vigorous reaction during heating. Shake gently to avoid excessive pressure buildup which could cause the solution to spray out and result in personal injury. If the sealing film breaks, promptly reseal with new sealing film.

3. Reagent III is highly irritating. It is recommended to handle all operations in a fume hood.

4. The amount of Reagent III used in the step where the supernatant is added can be adjusted according to the degree of acetylation of your sample, ensuring the absorbance value is between 0.1 and 0.8, and include this adjustment in the calculation formula.

5. Due to the volatility of glacial acetic acid, please measure immediately after mixing, and it is recommended to mix and measure one tube at a time.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly

equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well quartz plate (non-polystyrene material) calculation formula as below

Lignin content (mg/g dry weight)=ΔA÷ε÷d×$V_{Detection}$÷($V_{Supernatant}$×W÷$V_{Acetylation}$)**=4.368×ΔA÷W**

Where: ε: Lignin extinction coefficient, 23.35 mL/mg/cm; d: 96-well quartz plate (non-polystyrene material) diameter, 0.5 cm; $V_{Detection}$: Detection volume, 1 mL; $V_{Supernatant}$: Supernatant volume taken, 0.02 mL; W: Sample dry weight, g; $V_{Acetylation}$: Acetylation reaction volume, 1.02 mL.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

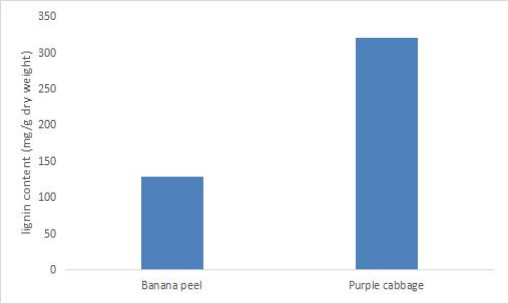

Figure 1. Determination lignin content in Banana peel and Purple cabbage by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.