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CheKine™ Mirco Cellulose (CLL) Concent Assay Kit

Cat #: KTB1720 Size: 48 T/48 S 96 T/96 S

	Mirco Cellulose (CLL) Concent Assay Kit		
REF	**Cat #:** KTB1720	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Cellulose (CLL) is a large polysaccharide molecule composed of glucose units, typically associated with hemicelluloses, pectins, and lignin, serving as the primary structural component of plant cell walls. It is a significant dietary fiber and the most abundant and widely distributed polysaccharide in nature. CheKine™ Mirco Cellulose (CLL) Concent Assay Kit offers a simple, convenient, and rapid approach for assessing CLL activity, which is suitable for plant tissue samples. The principle involves cellulose, a polymer of β-glucose residues, decomposing into β-glucose under acidic conditions upon heating. Under strong acid conditions, β-glucose can dehydrate to form β-furfural compounds. These β-furfural compounds then undergo dehydration condensation with anthrone to generate furfural derivatives. The depth of the resulting color can be indirectly quantified to determine the cellulose content.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	60 mL	120 mL	4℃, protected from light
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅲ	3 mL	6 mL	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	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 visible spectrophotometer capable of measuring absorbance at 620 nm
- Oven, vortex mixer, 40-mesh sieve, water bath, analytical balance, ice maker, low-temperature centrifuge
- 96-well plate (non-polystyrene material) or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water, 80% ethanol, acetone, concentrated sulfuric acid
- Dounce homogenizer

Reagent Preparation

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

Working Reagent II: Prepared before use. For a 48 T, add 2.8 mL of Reagent III, and for a 96 T, add 5.6 mL of Reagent III, mixing until fully dissolved. If dissolution is difficult, heat and stir the mixture. Unused reagent can be stored at 4℃ for up to one week.

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

Note: Reagent I, Reagent II and Reagent III are toxic and have a pungent odor, so it is recommended to experiment in a fume hood.

Standard: Prepared before use. Dissolve by adding 1 mL of deionized water to make a 10 mg/mL glucose solution, and reserve for later use. This solution can be kept at 4℃ for up to two weeks.

Standard preparation: Using 10 mg/mL glucose solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume	Deionized Water Volume (μL)	Concentration (mg/mL)
Std.1	25 μL 10 mg/mL Standard	975	0.25
Std.2	500 μL of Std.1 (0.25 mg/mL)	500	0.125
Std.3	500 μL of Std.2 (0.125 mg/mL)	500	0.0613
Std.4	500 μL of Std.3 (0.0613 mg/mL)	500	0.0306
Std.5	500 μL of Std.4 (0.0306 mg/mL)	500	0.0153
Std.6	500 μL of Std.5 (0.0153 mg/mL)	500	0.0077
Std.7	500 μL of Std.6 (0.0077 mg/mL)	500	0.0038
Std.8	0	500	0 （Blank Tube）

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.

1. Weigh fresh sample 0.3 g (recorded as W_1), dry at 80℃ for 2 h (extension may be appropriate) until constant weight, crush, and sieve through a 40 mesh. Add 1 mL of 80% ethanol to the dried sample, incubate in a 90 ℃ water bath for 20 min (recommended to seal with parafilm or use an EP tube with a screw cap), cool to room temperature, centrifuge at 8,000 g and 25℃ for 10 min, discard the supernatant. Wash the precipitate once each with 1.5 mL of 80% ethanol and acetone respectively (vortex for about 2 min, centrifuge at 8,000 g and 25℃ for 10 min, discard the supernatant), dry the precipitate, which then serves as crude cell wall, weigh and record as W_2.

2. Weigh 0.01 g of the above crude cell wall (recorded as W_3), add 1 mL of Reagent I, mix well, and incubate in a 90℃ water bath for 30 min (recommended to seal with parafilm or use an EP tube with a screw cap). After cooling, centrifuge at 8,000 g and 25℃ for 10 min, discard the supernatant. Add 1 mL of deionized water to the precipitate, mix, vortex for about 2 min, centrifuge at 8,000 g and 25℃ for 10 min, discard the supernatant, and repeat the washing process with deionized water three times (add 1 mL, vortex, centrifuge as before). Add 1 mL of acetone to the precipitate, centrifuge, discard the supernatant, and dry the precipitate for later use.

3. Dissolve the dried precipitate in 0.5 mL of deionized water (if not fully soluble, gentle homogenization or ultrasonication can be used to aid dissolution), place on ice-water bath, slowly add 0.75 mL of concentrated sulfuric acid, mix, let stand for 30 min on ice-water bath, take part of the supernatant, dilute 20 times with deionized water, centrifuge at 8,000 g and 4℃ for 10 min, and retain the supernatant for testing.

Note: For samples with low cellulose content (high starch content), such as rice flour, chestnuts, sweet potatoes, etc.

Dilution may be omitted or reduced.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 620 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Adjust the water bath to 95℃.
3. Operation table (The following operations are operated in a 1.5 mL EP tube):

Reagent	Blank Tube (μL)	Test Tube (μL)	Standard Tube (μL)
Supernatant	0	150	0
Standard	0	0	150
Deionized Water	150	0	0
Working Reagent II	35	35	35
Concentrated sulfuric acid	315	315	315

Mix well and place in a 95℃ water bath for 10 min (make sure it is tightly covered to prevent water evaporation). After cooling down to room temperature, take 200 μL of the upper layer liquid and transfer it to a micro glass cuvette or a 96-well plate (non-polystyrene material), then measure the absorbance at 620 nm, recording the values as A_{Blank}, A_{Test} and $A_{Standard}$. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If ΔA is less than 0.03, it is advisable to reduce the dilution ratio or increase the sample volume appropriately. If ΔA is greater than 1.0, the sample can be appropriately diluted with Deionized Water, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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.

1. Drawing of standard curve:

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve, get the standard equation, and bring the ΔA_{Test} into the equation to get the x value (mg/mL).

2. Calculation of CLL concent:

(1) Calculated by sample fresh weight

CLL (U/g fresh weight)=$x\times V_{Extract}\times 20\times(W_2\div W_3)\div W_1\div 1.11$**=$22.52\times x\times W_2\div W_3\div W_1$**

(2) Calculated by dry weight of cell wall material CLL

CLL (U/g dry weight)=$x\times V_{Extract}\times 20\div W_3\div 1.11$**=$22.52\times x\div W_3$**

Where: 1.11: The constant for converting glucose content, as measured by this method, to CLL content; meaning that 111 μg of glucose reacted with anthrone reagent gives a color equivalent to that produced by 100 μg of CLL with the anthrone reagent. $V_{Extract}$: Volume of CLL extraction solution, 1.25 mL (composed of 0.5 mL deionized water+0.75 mL concentrated sulfuric acid). 20: The dilution factor of the sample. W_1: Fresh weight of the sample, 0.3 g. W_2: Mass of the cell wall material in the sample, g. W_3: Mass of the cell wall material taken for CLL extraction, 0.01 g.

Precautions

1. If the sample or the dried precipitate is hard, it can be crushed first before proceeding with subsequent steps.
2. During the extraction of CLL, firstly, the EP tube immersed in the ice-water bath should be secured in place to avoid floating around; this ensures personal safety and prevents the entry of ice-water mixture into the EP tube, which could lead to

experimental errors. Furthermore, when adding concentrated sulfuric acid, it is recommended to immerse the pipette tip below the liquid surface of the sample and add it gradually to prevent boiling of the liquid surface and carbonization of the sample.

Typical Data

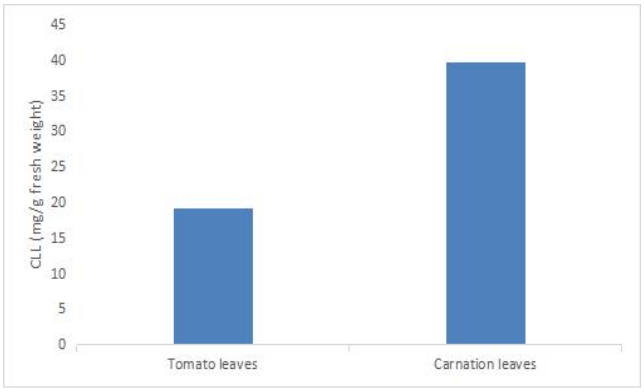

Figure 1. Determination CLL concent in Tomato leaves and Carnation leaves by this assay kit

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.