CheKine™ Mirco Hemicellulose Concent Assay Kit

Cat #: KTB1730 Size: 48 T/96 T

	Mirco Hemicellulose Concent Assay Kit
REF	**Cat #:** KTB1730 — LOT **Lot #:** Refer to product label
	Applicable sample: Plant Tissues
	Storage: Stored at 4℃ for 6 months, protected from light

Assay Principle

Hemicellulose is a mixture of polysaccharides that is tightly bound to cellulose in plant cell walls and is a major component of the primary cell wall. It is widely present in plants and represents a novel exploitable energy source. CheKine™ Mirco Hemicellulose Concent Assay Kit offers a simple, convenient, and rapid approach for assessing hemicellulose concent, which is suitable for plant tissue samples. The principle involves the conversion of hemicellulose into reducing sugars through acid hydrolysis. These reducing sugars then react with DNS (3,5-dinitrosalicylic acid) to form a reddish-brown compound that exhibits a characteristic absorption peak at 540 nm. The magnitude of the absorbance value is indicative of the amount of hemicellulose present.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	50 mL	100 mL	4℃
Reagent Ⅱ	50 mL	100 mL	4℃
Reagent Ⅲ	30 mL	60 mL	4℃
Reagent Ⅳ	2.5 mL	5 mL	4℃, protected from light
Standard	1	1	4℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm
- Oven, vortex mixer, 40-mesh sieve, water bath, analytical balance, ice maker, low-temperature centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water, 80% ethanol, acetone
- Dounce homogenizer

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Reagent Preparation

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

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℃.

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

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

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

Num.	Standard Volume	Deionized Water Volume (μL)	Concentration (mg/mL)
Std.1	50 μL 10 mg/mL Standard	450	1
Std.2	180 μL of Std.1 (1 mg/mL)	20	0.9
Std.3	160 μL of Std.1 (1 mg/mL)	40	0.8
Std.4	120 μL of Std.1 (1 mg/mL)	80	0.6
Std.5	80 μL of Std.1 (1 mg/mL)	120	0.4
Std.6	40 μL of Std.1 (1 mg/mL)	160	0.2
Std.7	20 μL of Std.1 (1 mg/mL)	180	0.1

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. Dry the sample at 80℃ until constant weight, crush, and sieve through a 40-mesh sieve. Weigh 0.01 g of the dried sample, add 1 mL of 80% ethanol, and incubate in a 90℃ water bath for 30 min. Cool to room temperature, centrifuge at 8,000 g and 25℃ for 10 min discard the supernatant and retain the precipitate, then dry at 80℃.
2. In the above dried precipitate, add 1 mL of Reagent Ⅰ, mix thoroughly, and incubate in a 95℃ water bath for 60 min. After cooling, centrifuge at 8,000 g and 25℃ for 10 min, discard the supernatant and retain the precipitate. Rinse with distilled water three times (add 1 mL of deionized water and vortex to mix), centrifuge at 8,000 g and 25℃ for 10 min, discard the supernatant and retain the precipitate. Rinse once with acetone (add 1 mL of acetone and vortex to mix), centrifuge at 8,000 g and 25℃ for 10 min, discard the supernatant and retain the precipitate, then dry at 80℃.
3. In the above dried precipitate, add 1 mL of Reagent Ⅱ, and incubate in a 95℃ water bath for 30 min. After cooling, centrifuge at 8,000 g and 25℃ for 10 min. Take 500 μL of the supernatant, add 500 μL of Reagent Ⅲ, mix well, and set aside for use.

Note: 1. The drying time at 80℃ is 2 h, which can be extended based on the sample condition.

2. During the heating processes at 90℃ or 95℃, the EP tubes may burst; it is recommended to seal the tubes with tape or use EP tubes with a screw cap that are explosion-resistant.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Adjust the water bath to 90℃.
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)

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Supernatant	0	60	0
Standard	0	0	60
Deionized Water	60	0	0
Reagent Ⅳ	195	195	195

Mix well and place in a 90℃ water bath for 5 min (make sure it is tightly covered to prevent water evaporation). Natural cooling, centrifuge at 8,000 g and 25℃ for 10 min, transfer 200 μL of the supernatant to a micro glass cuvette or a 96-well plate, and measure the absorbance at 540 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 0.2, it is advisable to reduce the dilution ratio or increase the sample volume appropriately. If ΔA is greater than 2.0, 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 y=kx+b, and bring the ΔA_{Test} into the equation to get the x value (mg/mL).

2. Calculation of hemicellulose concent:

Hemicellulose concent (mg/g dry weight)**$=x\times V_{Extract}\div W\times F$**

Where: $V_{Extract}$: Volume of extraction solution added, 2 mL. W: Dried sample mass, g; F: Sample dilution factor.

Typical Data

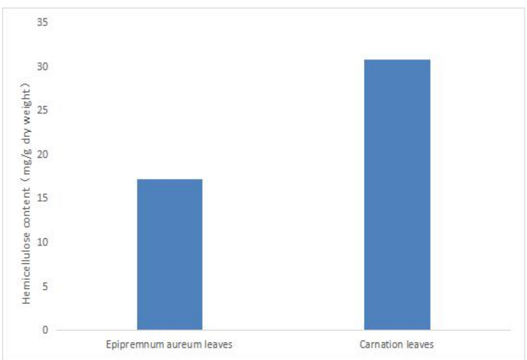

Figure 1. Determination hemicellulose concent in Epipremnum aureum leaves and Carnation leaves by this assay kit

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.

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