CheKine™ Micro Starch Assay Kit

Cat #: KTB1371 Size: 96 T/96 S

	Micro Starch Assay Kit		
REF	**Cat #:** KTB1371	LOT	**Lot #:** Refer to product label
	Detection range: 0.02-1 mg/mL (The detection range corresponds to the standard)		**Sensitivity:** 0.02 mg/mL (The sensitivity corres -ponds to the standard)
	Applicable sample: Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Starch is the main storage form of sugar in plants, and its content determination is of great significance for evaluating the nutritional value of food and investigating sugar metabolism in plants. Soluble sugar in the sample can be separated from starch by 80% ethanol, and starch can be further decomposed into glucose by acid hydrolysis, and the starch content can be calculated by measuring the glucose content by anthrone colorimetry.

Materials Supplied and Storage Conditions

Kit components	Size (96 T)	Storage conditions
Extraction Buffer	120 mL	4℃
Reagent Ⅰ	50 mL	4℃
Reagent Ⅱ	Powder×1 vial	4℃, protected from light
Standard	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
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Thermostatic incubator,thermostat water bath, centrifuge
- Deionized water, concentrated sulfuric acid
- mortar, sealing film

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

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

Reagent II: Prepared before use. After adding 5.25 mL of deionized water, slowly add 29.75 mL of concentrated sulfuric acid, continuously stir and dissolve thoroughly for use. It can be stored in dark at 4℃ for one week.

Note: Reagent I has a pungent odor, Reagent II is toxic, concentrated sulfuric acid is highly corrosive. so it is recommended to experiment in a fume hood.

Standard: Prepared before use. Add 1 mL of deionized water to dissolve it, resulting in a 10 mg/mL glucose standard solution that can be stored at 4℃ for 2 weeks.

Standard preparation: Use 10 mg/mL standard, prepare standard curve dilution as described in the table.

Num.	Standard Volume	Deionized Water Volume (μL)	Concentration (mg/mL)
Std.1	200 μL 10 mg/mL Standard	1,800	1
Std.2	400 μL of Std.1 (1 mg/mL)	600	0.4
Std.3	200 μL of Std.1 (1 mg/mL)	800	0.2
Std.4	100 μL of Std.1 (1 mg/mL)	900	0.1
Std.5	50 μL of Std.1 (1 mg/mL)	950	0.05
Std.6	40 μL of Std.1 (1 mg/mL)	960	0.04
Std.7	30 μL of Std.1 (1 mg/mL)	970	0.03
Std.8	20 μL of Std.1 (1 mg/mL)	980	0.02
Blank	0	1,000	0

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: 1. 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. 2. Some reagents have strong corrosiveness, and protection must be taken during operation.

1. After the plant tissue is dried at 37℃ or naturally air-dried, weigh about 0.03 g of the sample, fully grind it in a mortar, add 1mL of Extraction buffer, quickly homogenize it, then transfer it to 2 mL EP tube (cover tightly and wrap the nozzle with sealing film), extract it in water bath at 80℃ for 30 min, 3,000 g, centrifuge at 25℃ for 5 min, discard the supernatant and leave the precipitate.
2. Add 0.5 mL deionized water to the precipitate and put it in a 95℃ water bath for gelatinization for 15 min (cover tightly and wrap the nozzle with sealing film).
3. After cooling, add 0.35 mL Reagent I, put it in a 95℃ water bath and extract for 15 min (cover tightly and wrap the nozzle with sealing film), and oscillate for 3-5 times.
4. After cooling, add 0.85 mL deionized water, mix well, centrifuge 3,000 g at 25℃ for 10 min, and take the supernatant to be tested.

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. Operation table (The following operations are operated in the 1.5 mL EP tube):

Reagent	Test Tube (μL)	Standard Tube (μL)	Blank Tube(μL)
Sample supernatant	50	0	0

Standard	0	50	0
Deionized water	0	0	50
Reagent II	250	250	250

3. After mixing, put it in a 95℃ water bath for 15 min (cover tightly and wrap the nozzle with sealing film), naturally cool it to room temperature and mix it thoroughly. take 200 μL to determine the 620 nm absorbance value in a microglass cuvette or 96 well plate.The absorbance of test well, standard well, blank well were recorded as A_{Test}, $A_{Standard}$, and A_{Blank}. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Before the experiment, it is recommended to select 2-3 samples with large expected differences for pre experiment. If A_{Test} is greater than 1.0, the sample can be further diluted with extracting solution (according to the ratio of 0.35 mL Reagent I: 1.35 mL deionized water, it is prepared as required), and the calculated result is multiplied by the dilution factor, or the sample size for extraction can be reduced.If A_{Test} is less than A_{Blank}, the sample size can be appropriately increased.

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 starch content:

Starch content (mg/g)$=x\times V_{Sample}\div(W\times V_{Sample}\div V_{Total\ Sample})\div 1.11\times N$**$=x\times 1.53\div W\times N$**

V_{Sample}: sample volume added, 0.05 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1.7 mL; W: sample weight, g; 1.11: It is the constant that the glucose content measured by this method is converted into starch content, that is, the color of 111 μg of glucose with anthrone reagent is equivalent to the color of 100 μg of starch with anthrone reagent; N: the sample dilution factor.

Typical Data

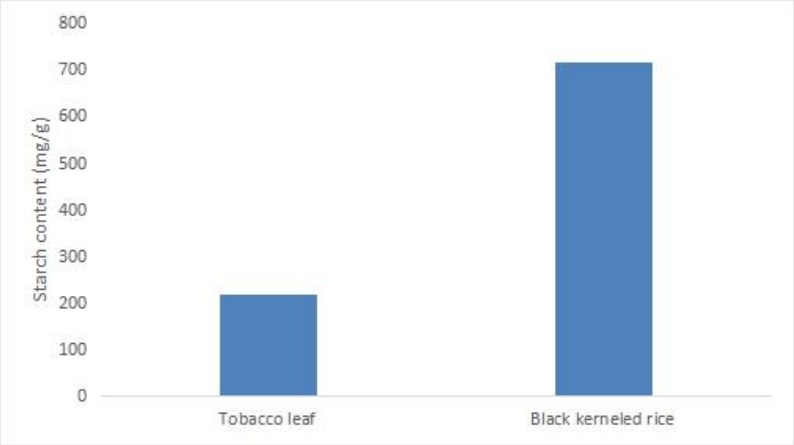

Figure 1. Determination starch content in tobacco leaf and black kerneled rice by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1100	CheKine™ Micro Lactic Acid（LA）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.