STARCH content kit

(Cat/No.:BC147 Size:50T/48S)

1. Principles of the Kit

The soluble sugar and starch in the sample can be separated by using 80% ethanol, the starch is further decomposed into glucose by acid hydrolysis, and then the glucose is quantified by anthrone colorimetry to calculate the content of related starch.

All reagents (sealed or not) should be kept at 2-8°C and remain stable for 12 months when sealed or for 1 month after unsealing.

2. The meaning of determination

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.

3. Compositions(The kit is valid for 6 months)

Reagent 1: 50mL×1 bottle, colorless transparent liquid, stored at 4° C;

Reagent 2: 30mL×1 bottle, colorless transparent liquid, store at 4° C;

Reagent 3: powder x 1 bottle, store at 4° C in the dark. Add 7.5mL of distilled water before use, then slowly add 42.5mL of concentrated sulfuric acid, stir while adding, fully dissolve, and store in the dark;

Reagent 4: standard product powder 10mg×1 bottle, store at 4° C;

0.5mg/mL standard substance: add 0.5mL distilled water and 6.5mL reagent 2 to the standard substance powder before use, shake and mix thoroughly, and dilute to 20mL with water to obtain the 0.5mg/mL standard substance application solution, store at 4° C.

0.05mg/mL standard product: Take the 0.5mg/mL standard product application solution and dilute it 10 times with distilled water to obtain the 0.05mg/mL standard product application solution.

4. Equipment or supplies requirement

Visible light spectrophotometer, desktop centrifuge, water bath, adjustable pipette, 1cm optical diameter cuvette, mortar, distilled water.

5. Procedures

1,Sample Preparation

(1) Accurately weigh 0.1g of sample, add 1mL of reagent-1, mechanically homogenate at 8000 rpm for 15 seconds, and repeat 3 times at intervals of 30 seconds. Extract in a water bath at 80°C for 20 minutes, centrifuge at 4000 rpm for 5 minutes, remove the supernatant and retain the precipitate;

(2) Add 0.5mL distilled water to the precipitate and gelatinize at 95°C for 15 minutes;

(3) After gelatinization, cool and stand still, add reagent 2 0.35mL, and bathe in 95℃ water for 10 minutes. (take out and mix once every 5 minutes);

(4) After completion, add 0.85mL distilled water, shake and mix well, centrifuge at 4000 rpm for 10 minutes, take the supernatant as the sample supernatant, and wait for the test.
(Sample supernatant generally needs to be diluted before performing the following operations. The specific dilution factor needs to be determined by taking individual samples for pre-testing. For example, potatoes are generally diluted 50-100 times with distilled water and then perform the following operations)

2,Measurement

	Blank tube	Standard	Sample
distilled water（mL）	0.2		
0.05mg/mL Standard solution（mL）		0.2	
Sample（mL）			0.2
Reagent III（mL）	1.0	1.0	1.0
After mixing, place in a water bath at 95°C for 10 minutes, cool naturally to room temperature, set the spectrophotometer to zero with distilled water at 620 nm, and measure the absorbance of each tube.			

6. Calculation

Starch content=$\frac{Asample-Ablank}{Astandard-Ablank}$ x $C_{standard}$ x V x N÷ W
mg/g tissue

$C_{standard}$: concentration of standard solution, 0.05mg/mL; V: total volume after sample pretreatment, 1.7mL; N: dilution ratio of sample supernatant; W: sample fresh weight, g.

Reference Value:
Example 1: Take 0.1g of fresh potato tissue and extract according to the steps. The supernatant of the sample is diluted 100 times with distilled water. The measured absorbance of the measuring tube is 0.662, the absorbance of the standard tube is 0.662, and the absorbance of the blank tube is 0.039. Substitute the data into the calculation formula:

Starch content=$\frac{0.462-0.039}{0.662-0.039}$ x 0.05x1.7 x 100÷0.1
g/100g tissue =57.71mg/g=5.771g/100g

Example 2: Take 0.1g of fresh edible pumpkin meat tissue and extract according to the steps. The supernatant of the sample is diluted 30 times with distilled water. The measured absorbance of the measuring tube is 0.479, the absorbance of the standard tube is 0.662, and the absorbance of the blank tube is 0.039. Substitute the data into the calculation formula:

Starch content=$\frac{0.479-0.039}{0.662-0.039}$ x 0.05x1.7 x 100÷0.1 =18.01mg/g=1.801g/mg
g/100g tissue

7. Notes

1. After homogenization, 1-2mL 80% ethanol can be used to clean the cutting head of the homogenizer, and the cleaned cleaning solution is incorporated into the sample homogenate solution for centrifugation;
2. Homogenize as fully as possible, otherwise the sugar in the larger particles cannot be separated and enter the supernatant of the sample to increase the measured value;
3. When operating, add the reagent three application solution as slowly as possible, otherwise it is easy to cause boiling and the liquid in the tube will rush out and hurt the human body;
4.
5. Use 2mL centrifuge tubes for operation, and do not need to pierce the holes when reacting at 95° C after adding the reagents and putting the lid on;
6. Mix before color comparison and then color comparison;
7. It is not necessary to make a standard curve, just perform single-point calibration according to the operation table (substituting into the calculation formula for calculation);
8. Be sure to wear gloves during the experiment to prevent the splash of sulfuric acid from harming the human body;
9. Sampling of dry samples can be less, such as sampling 0.01g ~ 0.02g, and the addition of other reagents remains unchanged.

Appendix: Preparation of Standard Curve

1. Pre-processing: Dilute the 0.5mg/mL standard solution with distilled water into S1 (0.00625mg/mL), S2 (0.0125mg/mL), S3 (0.025mg/mL), S4 (0.05mg/mL), S5 (0.1mg/mL) concentration to prepare the standard curve.

2. Measurement

No.	S0	S1	S2	S3	S4	S5
Standard solutions with different concentrations（mL）		0.2	0.2	0.2	0.2	0.2
distilled water（mL）	0.2					
Reagent III（mL）	1.0	1.0	1.0	1.0	1.0	1.0
After mixing, take a water bath at 95° C for 10 minutes, cool down to room temperature naturally, set the spectrophotometer to zero with distilled water at 620nm, and measure the absorbance value of each tube						

3. Test result

Standard concentration（mg/mL）	Absorbance values	absolute absorbance
0	0.039	0
0.00625	0.118	0.079
0.0125	0.193	0.154
0.025	0.348	0.309
0.05	0.662	0.623
0.1	1.257	1.218

Standard curve