Starch Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Cat No: BC0700

Size: 50T/48S

Components:

Reagent I: 50 mL×1. Storage at 4℃.

Reagent II: 20 mL×1. Storage at 4℃.

Reagent III: Powder×1. Storage at 4℃.

Standard: Powder×1, 10 mg glucose(anhydrous). Storage at 4℃. Standard solution: dissolved with 1 mL of distilled water to 10 mg/mL when the standard solution will be used.

Product Description:

Starch is the main storage form of sugar in plants. The determination of starch content has great significance in evaluating the nutritional value of food and researching the sugar metabolism in plants.

Separate soluble sugar from starch by 80% ethanol, then decompose starch into glucose by acid hydrolysis. The starch content can be calculated by measuring the glucose content using an anthrone colorimetric method.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, water-bath, adjustable pipette, 1 mL glass cuvette, mortar/homogenizer, ice, concentrated H_2SO_4 and distilled water.

Procedure:

I. Sample preparation:

1. Take 0.1 g of sample, grind in mortar and add 1 mL of Reagent I. After homogenization, transfer to a centrifuge tube, place in a water bath at 80℃ for 30 minutes, then centrifuge at room temperature and 3000 ×g for 5 minutes, discard the supernatant.
2. Add 0.5 mL of distilled water to the precipitate. Place in boiling water bath for 15 minutes (tighten the lid to prevent water loss)
3. After cooling, add 0.35 mL Reagent II, place at room temperature for15 min, shake 3~5 times.
4. Add 0.85 mL of distilled water. Mix well, centrifuge at room temperature and 3000 ×g for 10 minutes, take the supernatant for testing.
5. Take 100 μL of supernatant and add 700 μL of distilled water, that is to say, eight times dilution is carried out.

Note: After dilution, if the absorbance of sample exceeds 1.5 or less than 0.1, it is recommended that the sample can be properly diluted or condensed before measurement.

II. Determination procedure:

1. Preheat the spectrophotometer for 30 minutes, adjust wavelength to 620 nm and set zero with distilled water.
2. Adjust the water-bath to 95 °C.
3. Standard working solution: dilute the 10 mg/mL standard solution with distilled water to 0.1、0.05、0.04、0.03、0.02、0.01 mg/mL.
4. Working solution: add 13.5 mL of distilled water to Reagent III, slowly add 76.5 mL of concentrated H_2SO_4, stir constantly and fully dissolve.
5. Take 0.2 mL of Standard solution or 0.2 mL of distilled water (Blank control) and add 1 mL Working solution to a EP tube. Place in a 95℃ water bath for 10 minutes (tighten the lid to prevent water loss), natural cool to room temperature, determine absorbance of Standard solution (A_S) and Blank control(A_B) at 620 nm. Calculate $\Delta A = A_S - A_B$.
6. Sample test: Take 0.2 mL of sample add 1 mL of Working solution to a EP tube. Place in 95℃ water bath for 10 minutes (tighten the lid to prevent water loss), naturally cool to room temperature, determine absorbance of Test tube (A_T) at 620 nm. Calculate $\Delta A' = A_T - A_B$.

III. Calculations:

1. Create standard curve

Take glucose standard solution (0.1、0.05、0.04、0.03、0.02、0.01 mg/mL) as x-axis, ΔA as y-axis, to draw the standard curve and obtain the linear regression equation y=kx+b, substituting ΔA′ into the equation yields x(mg/mL).

2. Calculation of starch content

Calculated by the mass of sample

Starch content (mg/g mass) $= x \times D \times V_E \div W \div 1.11 = 12.252x \div W$

V_E: extraction volume, 1.7 mL; W: fresh sample weight, g; D： Dilution factor, 8. 1.11: is the constant of converting glucose content measured by this method into starch content, that is, 111 μg glucose is colored by anthrone reagent, which is equivalent to 100 μg starch by anthrone reagent.

Note:

1. As the working fluid is highly corrosive, please operate with caution.
2. If the absorbance value exceeds the linear range, the sample size can be increased or diluted before determination.

Experimental example:

1. Take 0.1g of willow for sample treatment, take the supernatant, and then operate according to the determination steps. Measure and calculate $\Delta A' = A_T - A_B = 0.905 - 0.097 = 0.808$, standard curve y=7.6785x+0.019, calculate x = 0.1028.

Starch content (mg/g mass) = x× dilution times × $V_E \div W \div 1.11 = 12.252x \div W = 12.252 \times 0.1028 \div 0.1$ = 12.595 mg/g mass.

Recent Products Citations：

[1] Moxian Chen,Fuyuan Zhu,Fengzhu Wang,et al. Alternative splicing and translation play important roles in hypoxic germination in rice. Journal of Experimental Botany. January 2019;(IF5.36)

[2] Kunyang Zhuang,Fanying Kong,Song Zhang,et al. Whirly1 enhances tolerance to chilling stress in tomato via protection of photosystem II and regulation of starch degradation. New Phytologist. October 2018;(IF7.299)

[3] Zexun Huai,Lishun Peng,Sheliang Wang,et al. Identification and Characterization of an Arabidopsis thaliana Mutant lbt With High Tolerance to Boron Deficiency. frontiers in plants science. June 2018;(IF4.106)

References：

[1] Clegg K M. The application of the anthrone reagent to the estimation of starch in cereals[J]. Journal of the Science of Food and Agriculture, 1956, 7(1): 40-44.

[2] Viles Jr F J, Silverman L. Determination of starch and cellulose with anthrone[J]. Analytical Chemistry, 1949, 21(8): 950-953.

Related Products：

BC0610/BC0615 Soil β-glucosidase(β- GC) Activity Assay Kit

BC2040/BC2045 β-amylase Activity Assay Kit

BC1850/BC1855 Soluble Starch Synthase(SSS) Activity Assay Kit