

## Starch Content Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Catalog Number:** BC0700

**Size:** 50T/48S

**Product Composition:** Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent Name	Size	Preservation Condition
Reagent I	Liquid 35mL×1	2-8°C
Reagent II	Liquid 35mL×1	2-8°C
Reagent III	Powder ×2	2-8°C
Standard	Powder ×1	2-8°C

### Solution Preparation:

**1. Standard:** Before use, add 1 mL distilled water to fully dissolve and prepare 10 mg/mL glucose standard solution for use; store the inexhaustible reagent at 2-8°C for 2 weeks.

**2. Preparation of working liquid:** Before use, take a bottle of Reagent III and add 6.75 mL distilled water, slowly add 38.25 mL concentrated sulfuric acid, stir continuously, fully dissolve, and wait for use, the reagent can be stored at 2-8°C for 1 week.

### 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.

### Technical Indicators:

**Minimum Detection limit:** 0.0027 mg/mL

**Linear Range:** 0.003-0.15 mg/mL

**Note:** Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorption value of the sample is not within the measurement range, it is recommended to dilute or increase the sample size for detection.

### Reagents and Equipment Required but Not Provided:

Spectrophotometer, water bath/metal bath, adjustable pipette, 1mL glass cuvette, mortar/homogenizer, ice, concentrated sulfuric acid (no express delivery allowed), distilled water.

### Procedure:

#### I. Sample preparation:

1. Take 0.03 g of sample, grind in mortar and add 0.6 mL of Reagent I. After homogenization,

transfer to

a centrifuge tube, place in a water bath at 80°C for 30 mins, then centrifuge at room temperature and 3000 ×g for 5 mins, discard the supernatant.

2. Add 0.3 mL of distilled water to the precipitate. Place in boiling water bath for 15 mins (Wrap the sealing film to prevent bursting).
3. After cooling, add 0.6 mL Reagent II, place in boiling water bath for 15 mins (Wrap the sealing film to prevent bursting), shake 3~5 times.
4. After cooling, centrifuge at 8000g at room temperature for 15min, and take the supernatant for testing. If there is still turbidity after centrifugation, the centrifugation can be repeated and the supernatant can be taken.

## 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 0.2, 0.1, 0.05, 0.04, 0.03, 0.02, 0.01mg/mL.
4. Standard test: Take 0.2 mL of standard solution or 0.2 mL of distilled water (Blank control) and add 1 mL working solution to an EP tube. Place in a 95°C 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  $\Delta A = A_S - A_B$ . The standard curve and blank tube only need to be done 1-2 times.
5. Sample test: Take 0.2 mL of sample add 1 mL of Working solution to a EP tube. Place in 95°C 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

According to the concentration of the standard tube ( $x$ , mg/mL) and the absorbance  $\Delta A$  standard ( $y$ ,  $\Delta A$  standard), establish a standard curve. According to the standard curve, substitute  $\Delta A'$  into the equation to get  $x$  (mg/mL).

2. Calculation of starch content

$$\text{Starch content (mg/g mass)} = x \times V_E \div W \div 1.11 \times F = 0.811x \div W \times F$$

$V_E$ : extraction volume, 0.9 mL;

$W$ : fresh sample weight, g;

$F$ : dilution ratio;

1.11: It is the constant of converting glucose content measured by this method into starch

content, that is, 111  $\mu\text{g}$  glucose is colored by anthrone reagent, which is equivalent to 100  $\mu\text{g}$  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 0.03g of wheat seeds for sample processing, take the supernatant, dilute it with distilled water 128 times for the experiment, and then follow the determination steps to measure and calculate  $\Delta A' = A_{\text{determination}} - A_{\text{blank}} = 0.929 - 0.106 = 0.823$ , the standard curve  $y = 4.9517x - 0.0756$ , calculate  $x = 0.181$ , and calculate the content according to the sample quality:  
Starch content (mg/g mass)  $= 0.811x \div W \times F = 0.8811 \times 0.181 \div 0.03 \times 128 = 626$  mg/g mass.

**Recent Products Citations:**

- [1] Guo D, Liu P, Liu Q, Zheng L, Liu S, Shen C, Liu L, Fan S, Li N, Dong J, Wang T. Legume-specific SnRK1 promotes malate supply to bacteroids for symbiotic nitrogen fixation. *Mol Plant*. 2023 Sep 4;16(9):1396-1412. doi: 10.1016/j.molp.2023.08.009. Epub 2023 Aug 19. PMID: 37598296.
- [2] Yang L, Liu P, Wang X, Jia A, Ren D, Tang Y, Tang Y, Deng XW, He G. A central circadian oscillator confers defense heterosis in hybrids without growth vigor costs. *Nat Commun*. 2021 Apr 19;12(1):2317. doi: 10.1038/s41467-021-22268-z. PMID: 33875651; PMCID: PMC8055661.
- [3] Liu H, Xiu Z, Yang H, Ma Z, Yang D, Wang H, Tan BC. Maize Shrek1 encodes a WD40 protein that regulates pre-rRNA processing in ribosome biogenesis. *Plant Cell*. 2022 Sep 27;34(10):4028-4044. doi: 10.1093/plcell/koac216. PMID: 35867001; PMCID: PMC9516035.
- [4] Xu J, Wang H, Xu R, Li Q, Su Y, Liu J, Zhu W. The diurnal fluctuation of colonic antibiotic resistome is correlated with nutrient substrates in a pig model. *Sci Total Environ*. 2023 Sep 15;891:164692. doi: 10.1016/j.scitotenv.2023.164692. Epub 2023 Jun 6. PMID: 37290656.
- [5] Wang H, Xu R, Li Q, Su Y, Zhu W. Daily fluctuation of colonic microbiome in response to nutrient substrates in a pig model. *NPJ Biofilms Microbiomes*. 2023 Nov 8;9(1):85. doi: 10.1038/s41522-023-00453-w. PMID: 37938228; PMCID: PMC10632506.

**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:**

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|---------------|---|
| BC0610/BC0615 | Soil $\beta$ -glucosidase( $\beta$ - GC) Activity Assay Kit |
| BC2040/BC2045 | $\beta$ -amylase Activity Assay Kit                         |
| BC1850/BC1855 | Soluble Starch Synthase(SSS) Activity Assay Kit             |