（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS！）

Catalog No：E-BC-K1209-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader(500-510 nm)

Detection range：0.01-1.00 mg/mL

Elabscience® Starch Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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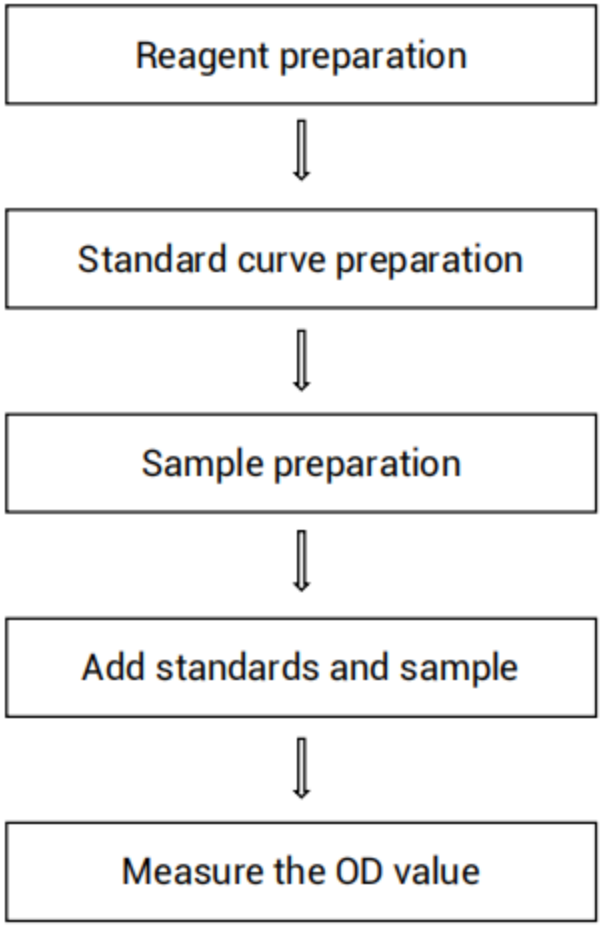
Assay summary



Intended use

The kit is suitable for detecting the content of soluble starch, resistant starch, and total starch in plant tissues.

Detection principle

Starch is a complex carbohydrate composed of numerous glucose units, it is classified into soluble starch and resistant starch.

Starch is enzymatically hydrolyzed to glucose, which subsequently reacts with a chromogenic agent to produce a red product. This colored compound exhibits a characteristic absorption peak at 505 nm, and the glucose concentration is directly proportional to the absorbance intensity at this wavelength.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Enzyme Buffer Solution	5.5 mL × 1 vial	11 mL × 1 vial	-20°C,12 months
Reagent 2	Enzyme Reagent	0.55 mL × 1 vial	1.1 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Chromogenic Buffer Solution	5.5 mL × 1 vial	11 mL × 1 vial	-20°C,12 months
Reagent 4	Chromogenic Agent A	0.11 mL × 1 vial	0.22 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Chromogenic Agent B	0.11 mL × 1 vial	0.22 mL × 1 vial	-20°C, 12 months, shading light
Reagent 6	Standard	Powder × 1 vial	Powder × 2 vials	-20°C,12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Microplate reader (500-510 nm, optimum wavelength: 505 nm), Incubator, drying oven, water bath

Materials required but not provided

Distilled or deionized water, 80% ethanol, 4 mol/L KOH、4 mol/L HCl, pH indicator paper

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② Enzyme Working Solution preparation:
Before testing, please prepare sufficient Enzyme Working Solution. For example, prepare 1000 μL of Enzyme Working Solution (mix well 900 μL of Enzyme Buffer Solution and 100 μL of Enzyme Reagent thoroughly). The Enzyme Working Solution should be freshly prepared before use. Stable for 8 h protected from light.

③ Chromogenic Working Solution preparation：
Before testing, please prepare sufficient Chromogenic Working Solution. For example, prepare 1000 μL of Chromogenic Working Solution (mix well 960 μL of Chromogenic Buffer Solution, 20 μL of Chromogenic Agent A and 20 μL of Chromogenic Agent B). The Chromogenic Working Solution should be freshly prepared before use. Stable for 8 h protected

from light.

④ 10 mg/mL Standard preparation:

Dissolve one vial of Standard with 1 mL of distilled or deionized water and heat in a boiling water bath for 5 minutes, stirring occasionally until completely dissolved. The 10 mg/mL Standard can be stable for 7 days when stored at 2-8°C protected from light. Before use, heat the 10 mg/mL standard in a boiling water bath for 5 minutes to ensure complete dissolution.

⑤ 1 mg/mL Standard preparation:

Before testing, please prepare sufficient 1 mg/mL Standard preparation. For example, prepare 1000 µL of 1 mg/mL Standard (mix well 900 µL of distilled or deionized water and 100 µL of 10 mg/mL standard). The 1 mg/mL Standard should be freshly prepared before use. Stable for 8 h.

⑥ Standard curve preparation:

Always prepare a fresh set of standards. Discard Working Standard Dilutions after use.

Dilute 1 mg/mL Standard with distilled or deionized water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 mg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (µmol/L)	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**1.0**
1 mg/mL Standard (µL)	0	20	40	60	80	120	160	200
distilled or deionized water (µL)	200	180	160	140	120	80	40	0

Sample preparation

Preparation of dry weight samples: Place the sample in an drying oven at 60-80°C and dry it until it reaches a constant weight, with the difference between the two weighings not exceeding 1 mg (alternative methods, such as freeze-drying, may also be used).

Soluble starch extraction:

Tissue sample:

① Homogenize 10 mg dry weight sample in 1 mL 80% ethanol with a dounce homogenizer.

② Heat the homogenate in a water bath at 80°C for 10 minutes, mixing every 2 minutes.

③ Centrifuge at 10000×g for 5 min. Discard the supernatant.

④ Add 1 mL of distilled or deionized wate to the precipitate, heat in a boiling water bath for 5 minutes.

⑤ Centrifuge at 10000×g for 2 min. Collect the supernatant for analysis and the precipitate is as the resistant starch extraction sample.

Resistant starch extraction:

Tissue sample:

① After extracting the soluble starch, add 1 mL of 4 mol/L KOH to the precipitate (after taking the supernatant) and mix thoroughly.

② Heat in a boiling water bath for 15 minutes.

③ Centrifuge at 10000×g for 2 min.

④ Transfer the supernatant to a new EP tube and add 1 mL of 4 mol/L HCl to adjust the pH to 7-8 (test 1 μL of the sample with pH paper) to prepare the resistant starch sample for testing.

⑤ If a precipitate forms in the sample during storage, dissolve it completely in a boiling water bath before testing.

Total starch extraction：

Tissue sample:

① Homogenize 10 mg dry weight sample in 1 mL 80% ethanol with a dounce homogenizer.

② Heat the homogenate in a water bath at 80°C for 10 minutes, mixing every 2 minutes.

③ Centrifuge at 10000×g for 5 min. Discard the supernatant.

④ Add 1 mL of 4 mol/L KOH to the precipitate.

⑤ Heat in a boiling water bath for 15 minutes.

⑥ Centrifuge at 10000×g for 2 min.

⑦ Transfer the supernatant to a new EP tube and add 1 mL of 4 mol/L HCl to adjust the pH to 7-8 (test 1 μL of the sample with pH paper) to prepare the Total starch sample for testing.

⑧ If a precipitate forms in the sample during storage, dissolve it completely in a boiling water bath before testing.

Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
1% dry corn seed tissue homogenization	1-3
1% dry potato tissue homogenization	2-5
1% dry wheat seed tissue homogenization	2-5
1% dry rice tissue homogenization	2-5

Note: The diluent is distilled or deionized water. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: Add 10 μL of Standard Solution with different concentrations to the corresponding wells.

Sample well: Add 10 μL of sample to the corresponding wells.

② Add 90 μL of Enzyme Working Solution into each well.

③ Shake the microplate for 5 seconds. Incubate at 37°C for 10 min.

④ Add 100 μL of Chromogenic Working Solution into each well.

⑤ Shake the microplate for 5 seconds. Incubate at 37°C for 10 min.

⑥ Measure the OD value of each well at 505 nm, as A.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the corrected OD value.

3. Plot the standard curve by using corrected OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Tissue sample:

$$\text{Soluble starch (mg/g dry weight)} = (\Delta A - b) \times V_1 \div m \times f$$

$$\text{Resistant starch (mg/g dry weight)} = (\Delta A - b) \times V_2 \div m \times f$$

$$\text{Total starch (mg/g dry weight)} = (\Delta A - b) \times V_2 \div m \times f$$

[Note]

ΔA: $\Delta F = F_{sample} - F_{blank}$

m: The dry weight of tissue, g.

V_1: The volume of distilled or deionized water added to the sample, mL.

V_2: The total volume of 4 mol/L KOH and 4 mol/L HCl added to the sample, mL.

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three dry potato samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/mL)	0.250	0.539	0.754
%CV	2.4	0.9	1.5

Inter-assay Precision

Three dry potato samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/mL)	0.137	0.341	0.488
%CV	7.5	3.5	3.0

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mg/mL)	0.250	0.550	0.850
Observed Conc. (mg/mL)	0.249	0.542	0.836
Recovery rate (%)	100%	99%	98%

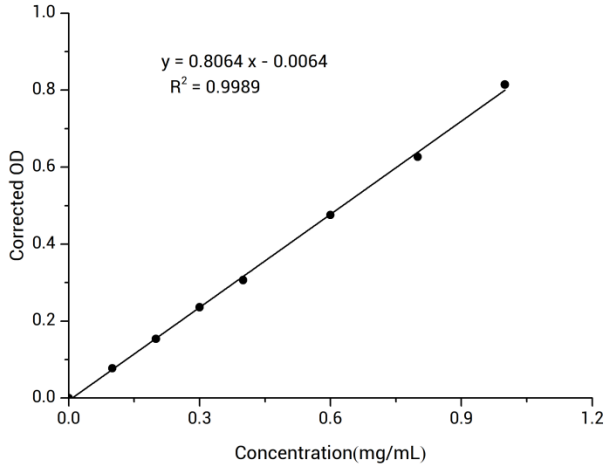
Sensitivity

The analytical sensitivity of the assay is 0.01 mg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mg/mL)	0	0.1	0.2	0.3	0.4	0.6	0.8	1.0
OD	0.050	0.126	0.201	0.286	0.362	0.516	0.663	0.878
	0.050	0.129	0.207	0.286	0.351	0.535	0.690	0.851
Average OD	0.050	0.128	0.204	0.286	0.357	0.526	0.677	0.865
Corrected OD	0	0.078	0.154	0.236	0.307	0.476	0.627	0.815



Appendix Π Example Analysis

Example analysis：

Take 10 μL of the 3-fold diluted 1% dry potato tissue homogenate to the well of microplate. Proceed according to the operating steps. The results are as follows:

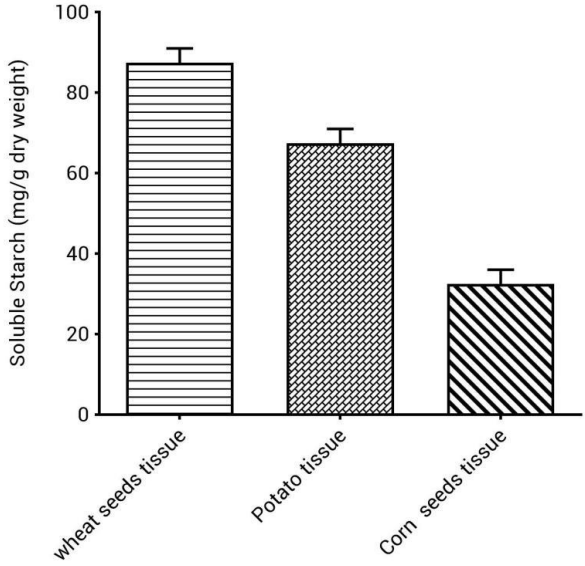
standard curve: y = 0.8064 x - 0.0064, the average OD value of the soluble starch sample well is 0.226, the average OD value of the resistant starch sample well is 0.560, the average OD value of the total starch sample well is 0.685, the average OD value of the blank well is 0.050, and the calculation result is:

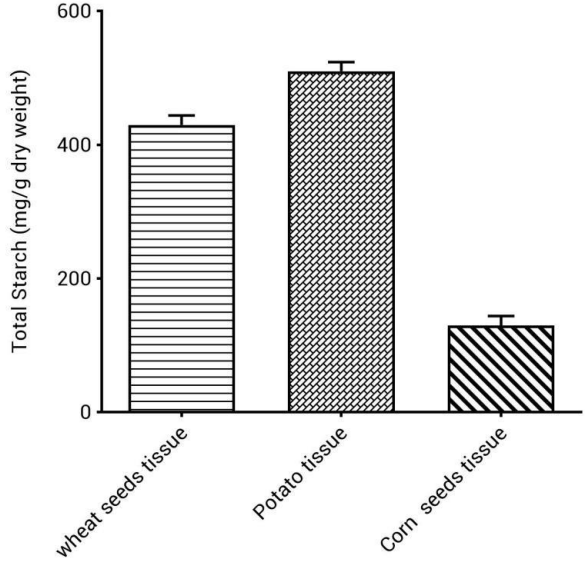
Soluble starch content (mg/g dry weight) = (0.226 - 0.050 + 0.0064) ÷ 0.8064 × 1 ÷ 0.01 × 3 = 67.86 mg/g dry weight

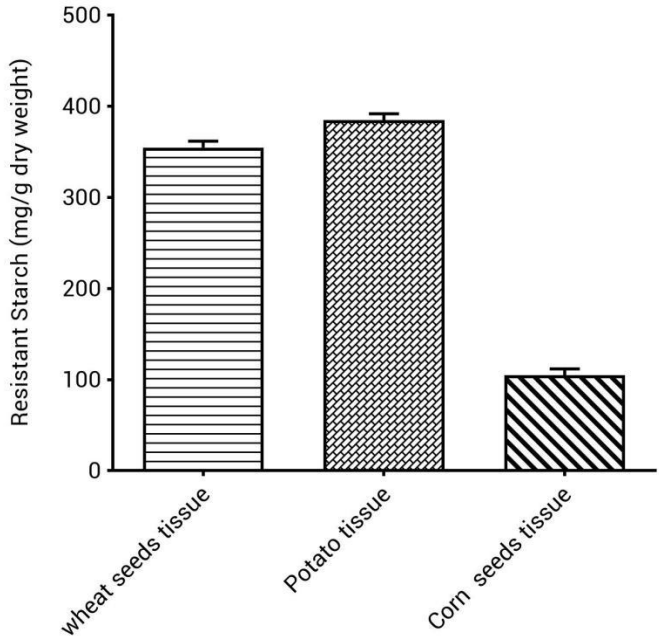
Resistant starch content (mg/g dry weight) = (0.560 - 0.050 + 0.0064) ÷ 0.8064 × 2 ÷ 0.01 × 3 = 384.23 mg/g dry weight

Total starch content (mg/g dry weight) = (0.685 - 0.050 + 0.0064) ÷ 0.8064 × 2 ÷ 0.01 × 3= 477.23 mg/g dry weight

Detect 1% dry wheat seeds tissue (dilute for 3 times), 1% dry sweet potato (dilute for 3 times), 1% dry corn seeds tissue (dilute for 3 times) according to the protocol, the result is as follows:


Soluble Starch (mg/g dry weight)
100
80
60
40
20
0
wheat seeds tissue
Potato tissue
Corn seeds tissue


Total Starch (mg/g dry weight)
600
400
200
0
wheat seeds tissue
Potato tissue
Corn seeds tissue


Resistant Starch (mg/g dry weight)
500
400
300
200
100
0
wheat seeds tissue
Potato tissue
Corn seeds tissue

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.