

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

Catalog No: E-BC-K1200-M

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

Measuring instrument: Microplate reader

(Detection wavelength:600 nm; Reference wavelength: 460 nm)

Detection range: 0.07-1 mg/mL

Elabscience[®] Amylose 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.

Table of contents

Assay summary	3
Intended use	4
Detection principle	4
Kit components & storage	4
Materials prepared by users	5
Reagent preparation	5
Sample preparation	6
The key points of the assay	7
Operating steps	7
Appendix I Performance Characteristics	9
Appendix II Example Analysis	11
Statement	12

Assay summary



Intended use

This kit can be used to measure the amylose content in plant tissue samples.

Detection principle

Amylose can be combined with the chromogenic component at 600 nm for maximum absorption, and the dual-wavelength method can be used to determine the content of amylose more accurately.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution A	50 mL × 1 vial	50 mL × 2 vials	2-8°C, 12 months
Reagent 2	Extracting Solution B	50 mL × 1 vial	50 mL × 2 vials	2-8°C, 12 months
Reagent 3	Saccharifying Reagent	55 mL × 1 vial	55 mL × 2 vials	2-8°C, 12 months
Reagent 4	Chromogenic Agent A	1 mL × 1 vial	2 mL × 1 vial	2-8°C, 12 months
Reagent 5	Chromogenic Agent B	1.2 mL × 1 vial	1.2 mL × 2 vials	2-8°C, 12 months shading light
Reagent 6	Standard	Powder × 1 vial	Powder × 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (Detection wavelength: 600 nm; Reference wavelength: 460 nm), Water bath, Vortex mixer

Reagents:

Ultrapure water

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of chromogenic working solution:

For each well, prepare 20 μL of chromogenic working solution (mix well 10 μL of chromogenic agent A and 10 μL of chromogenic agent B). The chromogenic working solution should be prepared on spot.

③ The preparation of 10 mg/mL standard solution:

Dissolve standard with 1000 μL of saccharifying reagent, mix fully, incubate it at 90°C water-bath for 10min, then cool down and mix well. Store at 2-8°C for 2 weeks.

④ The preparation of 1 mg/mL standard solution:

Before testing, please prepare sufficient 1 mg/mL standard solution. For example, prepare 1000 μL of 1 mg/mL standard solution (mix well 100 μL of 10 mg/mL standard solution and 900 μL of saccharifying reagent). Store at 2-8°C for 3 days.

⑤ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 mg/mL standard solution with saccharifying reagent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.4, 0.5, 0.7, 0.8, 1.0 mg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/mL)	0	0.1	0.2	0.4	0.5	0.7	0.8	1.0
1 mg/mL standard (μL)	0	20	40	80	100	140	160	200
Saccharifying reagent (μL)	200	180	160	120	100	60	40	0

Sample preparation

Tissue sample:

- ① Dry and grind the sample thoroughly (drying at 80°C to constant weight, and the difference between the two weighing is not more than 1 mg).
- ② Harvest the amount of tissue needed for each assay (initial recommendation 10 mg)
- ③ Homogenize 10 mg tissue in 1 mL of extracting solution A with a dounce homogenizer at 4°C. Incubate at 80°C water-bath for 30min, then cool down with running water.
- ④ Centrifuge 5000×g at 25°C for 5 min, discard the supernatant, and collect precipitate. Dissolve the precipitate with 1 mL of extracting solution B, shake and mix well for 5 min.
- ⑤ Centrifuge 5000×g at 25°C for 5 min, discard the supernatant, and collect precipitate. Add 1 mL of saccharifying reagent, mix fully. Incubate at 90°C water-bath for 10 min for glycosylation, then cool down with running water.

② Dilution of sample

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

Sample type	Dilution factor
10% Potato tissue homogenate	2-8
10% Corn tissue homogenate	2-8
10% Rice tissue homogenate	2-8
10% Edible starch tissue homogenate	2-10

Note: The diluent is saccharifying reagent. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

- ① It's better to measure no more than 20 sample wells at same time.
- ② If precipitation is still observed in the sample or standard, the mashing time (90°C water bath time) can be appropriately extended to ensure adequate mashing, so as not to affect the test results.
- ③ The experiment involves a long time high temperature water bath, which may cause volume loss of the system. Please ensure that the container is well sealed. If the solution volume is loss, the volume of the system is added to 1 mL with the reagent added before heating.

Operating steps

- ① Standard well: add 50 μ L of standard with different concentrations into the standard wells.
Sample well: add 50 μ L of sample into the sample wells.
- ② Add 20 μ L of chromogenic working solution into each well.
- ③ Add 180 μ L of ultrapure water into each well.
- ④ Mix well with microplate reader. Measure the OD values of each well at 600 nm with microplate reader, as A_1 ; measure the OD values of each well at 460 nm with microplate reader, as A_2 , $\Delta A = A_1 - A_2$.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean ΔA value of the blank (Standard # ①) from all standard readings. This is the absolved ΔA value.
3. Plot the standard curve by using absolved ΔA 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:

1. Tissue sample:

$$\text{amylose content (mg/g)} = (\Delta A_{\text{sample}} - \Delta A_{\text{blank}} - b) \div a \times V \times f \div m$$

[Note]

ΔA_{sample} : $\Delta A_{\text{sample}} = A_1 - A_2$

ΔA_{blank} : The ΔA value when the standard concentration is 0.

V: The volume of saccharifying reagent in preparation of sample, 1 mL

m: The weight of the sample, 0.01g.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three corn tissue 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)	0.25	0.50	0.75
%CV	6.1	5.0	2.6

Inter-assay Precision

Three corn tissue 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)	0.25	0.50	0.75
%CV	7.3	5.2	6.0

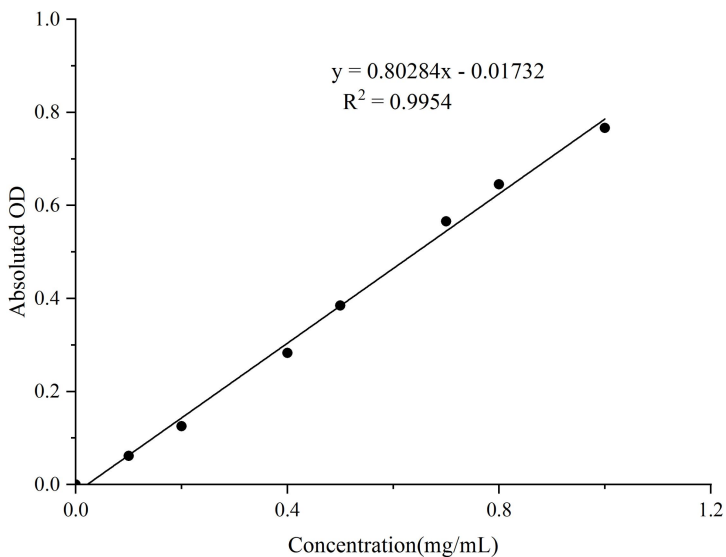
Sensitivity

The analytical sensitivity of the assay is 0.02 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	0.4	0.5	0.7	0.8	1.0
A₁	0.038	0.210	0.358	0.658	0.849	1.180	1.294	1.550
	0.042	0.211	0.361	0.679	0.830	1.181	1.331	1.579
Average A₁	0.040	0.211	0.360	0.669	0.840	1.181	1.313	1.565
A₂	0.070	0.184	0.269	0.413	0.493	0.664	0.701	0.826
	0.080	0.184	0.269	0.428	0.486	0.636	0.704	0.840
Average A₂	0.075	0.184	0.269	0.421	0.490	0.650	0.703	0.833
Average A₁ - A₂	-0.035	0.027	0.091	0.248	0.350	0.531	0.610	0.732
Absoluted ΔA	0.000	0.062	0.126	0.283	0.385	0.566	0.645	0.767



Appendix II Example Analysis

Example analysis:

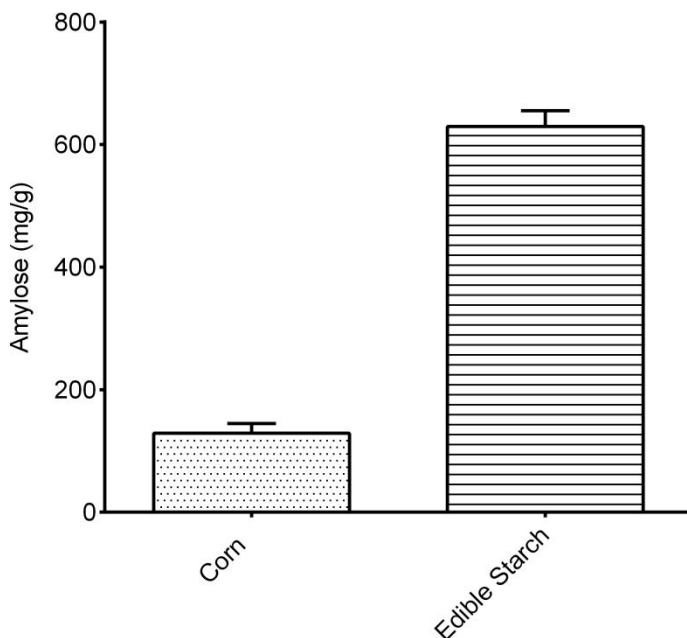
For corn sample, take the prepared sample and dilute for 8 times with saccharifying reagent, and carry the assay according to the operation steps. The results are as follows:

The standard curve: $y = 0.80284x - 0.01732$, the A_1 value of the sample well is 0.515, the A_2 value of the sample well is 0.434, the A_1 value of the blank well is 0.055, the A_2 value of the blank well is 0.090, and the calculation result is:

$$\text{amylose content (mg/g)} = (0.515 - 0.434 - 0.055 + 0.090 + 0.01732) \div 0.80284$$

$$\times 1 \times 8 \div 0.01 = 132.84 \text{ mg/g}$$

Detect corn tissue homogenate (dilute for 8 times) and edible starch homogenate (dilute for 10 times) according to the protocol, the result is as follows:



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.