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

**Catalog No：E-BC-K1201-M**

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

**Measuring instrument：Microplate reader**

**(Detection wavelength: 530 nm; Reference wavelength: 710 nm）**

**Detection range：0.012-1 mg/mL**

# **Elabscience®** Amylopectin Colorimetric Assay Kit

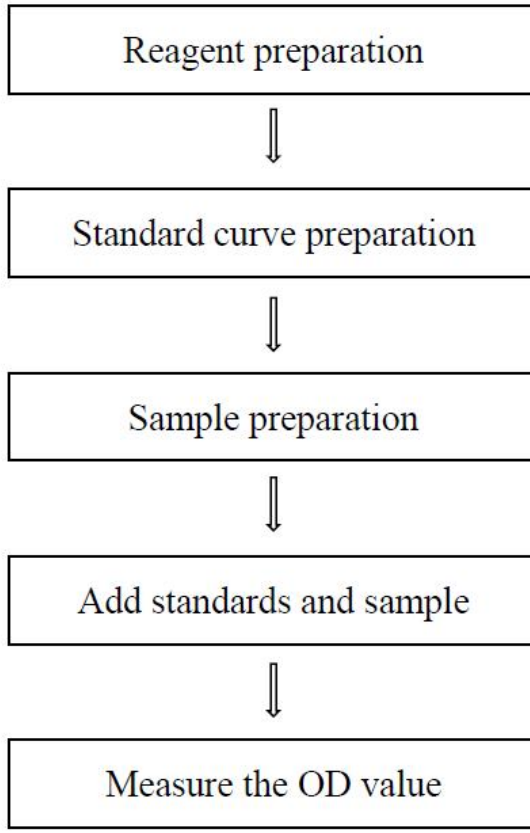
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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**



## Intended use

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

## Detection principle

Amylopectin can be combined with the chromogenic component at 530 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℃, 12 months |
| Reagent 2 | Extracting Solution B | 50 mL × 1 vial | 50 mL × 2 vials | 2-8℃, 12 months |
| Reagent 3 | Saccharifying Reagent | 55 mL × 1 vial | 55 mL × 2 vials | 2-8℃, 12 months |
| Reagent 4 | Chromogenic Agent A | 1 mL × 1 vial | 2 mL × 1 vial | 2-8℃, 12 months |
| Reagent 5 | Chromogenic Agent B | 1.2 mL × 1 vial | 1.2 mL × 2 vials | 2-8℃, 12 months shading light |
| Reagent 6 | Standard | Powder × 1 vial | Powder × 1 vial | 2-8℃, 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: 530 nm; Reference wavelength: 710 nm), Water bath, Vortex mixer

**Reagents:**

Ultrapure water

## Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of chromogenic working solution：

For each well, prepare 30 μL of chromogenic working solution (mix well 20 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℃ water-bath for 10 min，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℃ 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℃. Incubate at 80℃ water-bath for 30min, then cool down with running water.

④ Centrifuge 5000×g at 25℃ 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℃ for 5 min, discard the supernatant, and collect precipitate. Add 1 mL of saccharifying reagent, mix fully. Incubate at 90℃ 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 | 2-8 |

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℃ 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 μL of standard with different concentrations into the standard wells.

Sample well: add 50 μL of sample into the sample wells.

② Add 30 μL of chromogenic working solution into each well.

③ Add 170 μL of ultrapure water into each well.

④ Mix well with microplate reader. Measure the OD values of each well at 530 nm with microplate reader, as $A_1$; measure the OD values of each well at 710 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 absoluted ΔA value.

3. Plot the standard curve by using absoluted 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:**

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

**[Note]**

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

$\Delta A_{\text{blank}}$: The ΔA value when the standard concentration is 0.

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

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** | 3.6 | 2.3 | 1.8 |

### 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** | 7.2 | 7.0 | 6.6 |

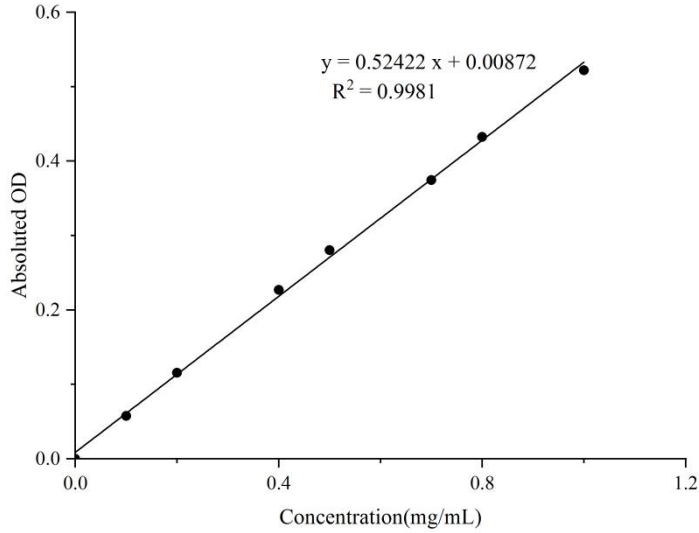
### Sensitivity

The analytical sensitivity of the assay is 0.012 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** |
|---|---|---|---|---|---|---|---|---|
| **$A_1$** | 0.067 | 0.141 | 0.219 | 0.359 | 0.428 | 0.550 | 0.620 | 0.733 |
| | 0.069 | 0.142 | 0.217 | 0.363 | 0.429 | 0.550 | 0.631 | 0.744 |
| **Average $A_1$** | 0.068 | 0.142 | 0.218 | 0.361 | 0.429 | 0.550 | 0.626 | 0.739 |
| **$A_2$** | 0.037 | 0.056 | 0.073 | 0.105 | 0.119 | 0.149 | 0.164 | 0.185 |
| | 0.041 | 0.054 | 0.074 | 0.105 | 0.119 | 0.144 | 0.164 | 0.190 |
| **Average $A_2$** | 0.039 | 0.055 | 0.074 | 0.105 | 0.119 | 0.147 | 0.164 | 0.188 |
| **Average $A_1$ - $A_2$** | 0.029 | 0.087 | 0.145 | 0.256 | 0.310 | 0.404 | 0.462 | 0.551 |
| **Absoluted ΔA** | 0.000 | 0.058 | 0.116 | 0.227 | 0.281 | 0.375 | 0.433 | 0.522 |



## Appendix II Example Analysis

**Example analysis：**

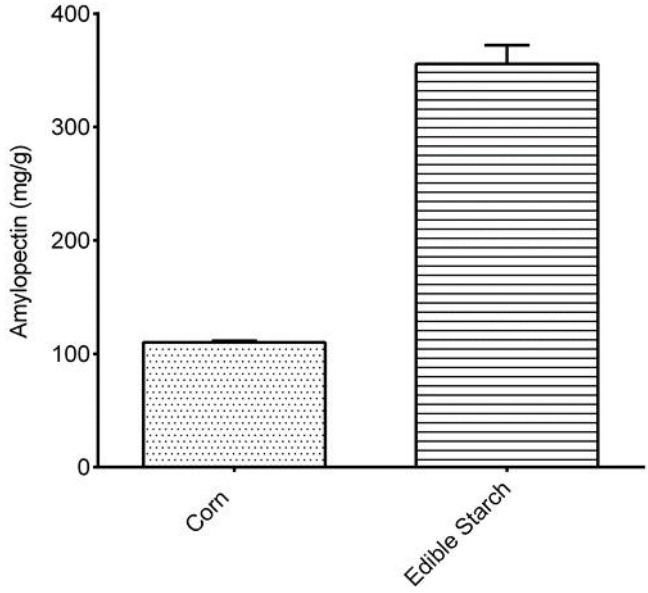
For corn sample, take the prepared sample and dilute for 8 times with saccharifying reagent, and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.52422x+0.00872, the $A_1$ value of the sample well is 0.423, the $A_2$ value of the sample well is 0.313, the $A_1$ value of the blank well is 0.068, the $A_2$ value of the blank well is 0.039, and the calculation result is:

amylopectin content（mg/g）$=(0.423-0.313-0.068+0.039-0.00872)\div 0.52422\times 1\times 8\div 0.01$

$= 110.30$ mg/g

Detect corn tissue homogenate (dilute for 8 times) and edible starch (dilute for 4 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.