Amylose Assay Kit

Catalog No.: abx294452

Size: 96 tests

Detection Range: 0.07 mg/ml – 1 mg/ml

Sensitivity: 0.02 mg/ml

Storage: Store all components at 4°C. Store the Chromogenic Reagent B in the dark.

Application: For detection and quantification of Amylose concentration in plant tissue homogenates.

Introduction

Amylose is polysaccharide that is important in plant energy storage. Amylose takes up less space than amylopectin due to its helical structure and is therefore the preferred starch for storage in pants. Amylose can also be used as a thickener, water binder, emulsion stabilizer, gelling agent, or act as a marker in laboratory settings.

Abbexa's Amylose Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Amylose concentration.This assay uses the dual-wavelength method to determine Amylose concentration. A Chromogenic Reagent is used which combines with the starch in the sample. OD measurements are recorded at 600 nm and 460 nm using a microplate reader and these values are used to calculate the concentration of Amylose in the sample.

Kit components

1. 96-well microplate
2. Chromogenic Reagent A: 2 ml
3. Chromogenic Reagent B: 2 × 1.2 ml
4. Standard: 1 vial
5. Extraction Solution A: 2 × 50 ml
6. Extraction Solution B: 2 × 50 ml
7. Saccharification Reagent: 2 × 55 ml
8. Plate sealer: 2

Materials required but not provided

1. Microplate reader (600 nm/ 460 nm)
2. Ultra-pure water
3. Pipette and pipette tips
4. 1.5 ml microcentrifuge tubes
5. Centrifuge
6. Vortex mixer
7. Water bath
8. Mechanical homogenizer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation method is intended as a guide and may be adjusted as required depending on the specific samples used.

- **Plant Tissue Homogenates:** Collect, dry, and grind the plant tissue sample. Drying should be carried out at 80°C. Record the mass of the sample periodically throughout the drying process. Stop drying the sample when the difference between the two recorded masses is < 1 mg. The recommended sample mass is 10 mg.

  Per 10 mg of sample, add into 1 ml of Extraction Solution A and homogenize manually, using a mechanical homogenizer, at 4°C. Incubate the sample in a 80°C water bath for 30 minutes, then cool with running water. Centrifuge the sample at 5000 × g for 5 minutes at 25°C. Collect the precipitate and discard the supernatant. Take the precipitate and add into 1 ml of Exctraction Solution B to dissolve. Vortex to mix thoroughly for 5 minutes. Centrifuge the sample at 5000 × g for 5 minutes at 25°C. Collect the precipitate and discard the supernatant. Take the precipitate and add into 1 ml of Saccharification Reagent. Vortex to mix thoroughly. Incubate the sample in a 90°C water bath for 10 minutes for glycosylation. Cool with running water and take the sample for assaying.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Saccharification Reagent, then carry out the assay procedure. 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:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for tissue homogenates.

2. Reagents

- **Chromogenic Working Solution:** Prepare enough Chromogenic Working Solution for the amount of wells to be used. Per well, add 10 µl of Chrmomogenic Reagent A to 10 µl of Chromogenic Reagent B and mix thoroughly to prepare 20 µl of Chromogenic Working Solution. Prepare immediately before assay.
- **Standard Stock Solution (10 mg/ml):** Dissolve 1 vial of Standard in 1 ml of Saccharification Reagent and mix thoroughly. Incubate in a 90°C water bath for 10 minutes, then cool with running water and mix thoroughly. Unused Standard Solution (10 mg/ml) can be aliquoted and stored at 2-8°C for up to 2 weeks.
- **Standard Working Solution (1 mg/ml):** Prepare enough Standard Working Solution (1 mg/ml) for the amount of wells to be used. To prepare 1 ml of Standard Working Solution (1 mg/ml) add 100 µl of Standard Stock Solution (10 mg/ml) to 900 µl of Saccharification Reagent and mix thoroughly. Unused Standard Working Solution (1 mg/ml) can be aliquoted and stored at 2-8°C for up to 3 days.
- **Standards:** Label 7 tubes with 1.0 mg/ml, 0.8 mg/ml, 0.7 mg/ml, 0.5 mg/ml, 0.4 mg/ml, 0.2 mg/ml, and 0.1 mg/ml. Add 200 µl, 160 µl, 140 µl, 100 µl, 80 µl, 40 µl, and 20 µl of Standard Working Solution (1 mg/ml) to the 1 mg/ml, 0.8 mg/ml, 0.7 mg/ml, 0.5 mg/ml, 0.4 mg/ml, 0.2 mg/ml, and 0.1 mg/ml tubes respectively, followed by 0 µl, 40 µl, 100 µl, 120 µl, 160 µl, 180 µl, and 200 µl of Saccharification Reagent, to prepare Standard Dilutions with concentrations 1 mg/ml, 0.8 mg/ml, 0.7 mg/ml, 0.5 mg/ml, 0.4 mg/ml, 0.2 mg/ml, and 0.1 mg/ml. These volumes are summarized in the following table:

Standard Dilution (mg/ml)	**1.0**	**0.8**	**0.7**	**0.5**	**0.4**	**0.2**	**0.1**
1 mg/ml Standard Working Solution (µl)	200	160	140	100	80	40	20
Saccharification Reagent (µl)	0	40	60	100	120	160	180

For the blank, or 0 mg/ml standard, use pure Saccharification Reagent. The volume of each standard will be 200 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- It is recommended to measure no more than 20 sample wells at one time.
- If precipitate is observed in the sample or standard after preparation, incubate futher in a 90°C water bath. Any precipitate present in the sample or standard may affect results.
- Ensure tubes are well sealed during the incubation steps to avoid loss of liquid volume. If liquid volume is lost during the incubation step, adjust to 1 ml using the reagent that was added before the incubation step.

B. Assay Procedure

1. Assign and record microplate well positions for each sample, standard, and blank.
2. Add 50 µl of sample into each sample well.
3. Add 50 µl of each standard dilution into the corresponding standard wells.
4. Add 50 µl of Saccharification Reagent into the blank well.
5. Add 20 µl of Chromogenic Working Solution into each well.
6. Add 180 µl of ultra-pure water into each well. Mix thoroughly.
7. Measure the OD of each well with a microplate reader at 600 nm. Record these values as A_1.
8. Measure the OD of each well with a microplate reader at 460 nm. Record these values as A_2.

C. Calculation of Results

Plot the standard curve, using the ΔA of the standard dilutions (adjusted for the ΔA of the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Amylose in each sample well can be derived with the following formula:

1. Tissue samples:

$$\text{Amylose Concentration (mg/g)} = \frac{(\Delta A_{\text{Sample}} - \Delta A_{\text{Blank}} - \text{b})}{\text{a}} \times \frac{V \times f}{W}$$

where:

ΔA_{Sample}	$A_{1\,\text{Sample}} - A_{2\,\text{Sample}}$
ΔA_{Blank}	$A_{1\,\text{Blank}} - A_{2\,\text{Blank}}$
V	Volume of Saccharification Reagent used in sample preparation (1 ml)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
W	The weight of the tissue sample (0.01 g)
F	The dilution factor of sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.