Beta-Amylase Assay Kit

Catalog No.: abx097990

Size: 96 tests

Detection Range: 0.97 U/g – 34.74 U/g

Sensitivity: 0.97 U/g

Storage: Store all components at 4°C for up to 12 months. Store the Chromogenic Reagent in the dark.

Application: For detection and quantification of Beta-Amylase activity in plant tissue homogenates.

Introduction

Beta-Amylase is an enzyme that hydrolyzes alpha-D-glucosidic linkages in polysaccharides such as starch. It is found in many bacteria, fungi, and plants, and plays a central role in the degradation of starch during the germination or malting of cereal grains. It has many biotechnological applications, such as the production of high maltose syrups.

Abbexa's Beta-Amylase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Beta-Amylase activity. In this assay, Beta-Amylase activity is calculated indirectly by measuring the activity of all amylases present in a sample, and the activity of Alpha-Amylase (the other major amylase present in plants). The difference in activity between these sample groups will be the Beta-Amylase activity. Beta-Amylase is highly thermolabile, and will denature at 70°C. Alpha-Amylase, in contrast, does not denature at temperatures this low. By exploiting this difference, the Alpha-Amylase can be isolated, and its activity can measured alone.
In the presence of starch, amylases catalyze the production of reducing sugars. 3,5-dinitrosalcylic acid is added to the samples being tested, which reacts with these reducing sugars to produce a brown-red compound, with an absorbance maximum at 540 nm. The intensity of the color is proportional to the amylase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Substrate: 10 ml
3. Chromogenic Reagent: 20 ml
4. Standard (10 mg/ml): 1.5 ml
5. Plate sealer: 2

Materials required but not provided

1. Microplate reader (540 nm)
2. Distilled water
3. Pipette and pipette tips
4. 1.5 ml microcentrifuge tubes
5. Centrifuge
6. Vortex mixer
7. Incubator

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 methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Carefully weigh 0.1 g of tissue, and add into 0.9 ml Distilled water. Homogenize manually, using a mechanical homogenizer or by ultrasonication, in an ice water bath. Collect the tissue homogenate, and stand at room temperature for 15 minutes, shaking every 5 minutes. Centrifuge at 3000 x g for 10 minutes, then carefully remove the supernatant and dilute with Distilled water for a final volume of 10 ml. Mark this as the *Alpha-Amylase Test Sample.*

  Aliquot 1 ml of the Alpha-Amylase Solution, and dilute with 4 ml of Distilled water. Mix fully. Mark this as the *Total Amylase Test Sample*.

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 Distilled water, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
1% *Epipremnum aurem* tissue homogenate	1
1% green pepper tissue homogenate	1
1% Maize grain tissue homogenate	1
1% *Daucus carota* tissue homogenate	1

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

- **Substrate** and **Chromogenic Reagent**: Preheat the Substrate and Chromogenic Reagent to 40°C, 10 minutes before starting the assay.
- **Standards:** Label 7 tubes with 1.4 mg/ml, 1.2 mg/ml, 1.0 mg/ml, 0.8 mg/ml, 0.6 mg/ml, 0.4 mg/ml, and 0.2 mg/ml. Add 140 µl, 120 µl, 100 µl, 80 µl, 60 µl, 40 µl, and 20 µl of Standard Solution (10 mg/ml) to the 1.4 mg/ml, 1.2 mg/ml, 1.0 mg/ml, 0.8 mg/ml, 0.6 mg/ml, 0.4 mg/ml, and 0.2 mg/ml tubes respectively, followed by 860 µl, 880 µl, 900 µl, 920 µl, 940 µl, 960 µl, and 980 µl of Distilled water, to prepare the Standard Dilutions with concentrations 1.4 mg/ml, 1.2 mg/ml, 1.0 mg/ml, 0.8 mg/ml, 0.6 mg/ml, 0.4 mg/ml, and 0.2 mg/ml. These volumes are summarized in the following table:

Standard Dilution (mg/ml)	**1.4**	**1.2**	**1.0**	**0.8**	**0.6**	**0.4**	**0.2**
10 mg/ml Standard (µl)	140	120	100	80	60	40	20
Distilled water (µl)	860	880	900	920	940	960	980

For the blank, or 0 mg/ml standard, use pure Distilled water. The volume of each standard will be 1000 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- If there is any precipitate present in the Substrate or Chromogenic Reagent, heat the vial to 70°C and stir gently to dissolve the precipitate. Allow the tubes to cool back to room temperature before use.
- Generally, absorbance values of samples tested should be < 0.747. If higher values are observed, dilute the sample before testing.
- If there is any precipitate in the final test supernatant, before testing centrifuge at 4000 × g for 5 minutes.

B. Assay Procedure

1. Mark microcentrifuge tubes for each standard, Alpha-Amylase sample and control, and Total Amylase sample and control. Each sample requires a corresponding control. The Alpha-Amylase and Total Amylase Test samples each require their own control. *It is strongly recommended to prepare all tubes in duplicate.*
2. Add 75 µl of each Alpha-Amylase Test Sample to each Alpha-Amylase sample tube, and 75 µl of the same sample to its corresponding control tube.
3. Incubate the samples and controls at 70°C for 15 minutes, then quickly cool with running water.
4. Add 75 µl of Total Amylase Test Sample to each Total Amylase sample tube, and 75 µl of the same sample to its corresponding control tube.
5. Add 75 µl of Substrate to all of the sample tubes (Alpha-Amylase and Total Amylase).
6. Add 75 µl of Distilled water to all of the control tubes (Alpha-Amylase and Total Amylase).
7. Incubate the sample and control tubes at 40°C for 5 minutes.
8. Add 75 µl of each standard dilution to the corresponding marked tubes.
9. Add 75 µl of Substrate to each standard tube.
10. Add 150 µl of Chromogenic Reagent to **all** of the sample, standard, and control tubes.

11. Mix fully, then incubate all tubes at 95°C for 5 minutes. Cool the tubes with running water and aliquot 250 µl of supernatant into the microplate. *Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
12. Measure the OD of each well with a microplate reader at 540 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions 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 Beta-Amylase in each sample well can be derived with the following formulae:

1. Total Protein

One unit of Beta-Amylase activity is defined as the amount required for 1 mg of tissue protein to produce 1 mg of reducing sugar per minute.

Total protein content in the sample needs to be assessed separately.

$$\text{Alpha-Amylase (U/mg protein)} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}} - \text{b}) \times \text{V}_{\text{Substrate}}}{\text{a} \times \text{t} \times \text{V}_{\text{Sample}} \times \text{C}_{\text{Protein}}}$$

$$\text{Total Amylase (U/mg protein)} = \text{F}_2 \times \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}} - \text{b}) \times \text{V}_{\text{Substrate}}}{\text{a} \times \text{t} \times \text{V}_{\text{Sample}} \times \text{C}_{\text{Protein}}}$$

2. Sample Weight

One unit of Beta-Amylase activity is defined as the amount required for 1 g of tissue to produce 1 mg of reducing sugar per minute.

$$\text{Alpha-Amylase (U/g fresh tissue)} = \text{F}_1 \times \frac{\text{V}_{\text{Tissue}}}{\text{V}_{\text{Sample}}} \times \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}} - \text{b}) \times \text{V}_{\text{Substrate}}}{\text{a} \times \text{t} \times \text{W}}$$

$$\text{Total Amylase (U/g fresh tissue)} = \text{F}_1 \times \text{F}_2 \times \frac{\text{V}_{\text{Tissue}}}{\text{V}_{\text{Sample}}} \times \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}} - \text{b}) \times \text{V}_{\text{Substrate}}}{\text{a} \times \text{t} \times \text{W}}$$

$$\text{Beta-Amylase activity} = \text{Total Amylase activity} - \text{Alpha-Amylase activity}$$

where:

OD_{Sample}	OD value of sample
$OD_{Control}$	OD value of corresponding control
$V_{Substrate}$	Volume of sample + Substrate (0.15 ml)
V_{Sample}	Volume of sample added to the microplate (0.075 ml)
V_{Tissue}	Volume of tissue preparation (10 ml)
$C_{Protein}$	Concentration of protein in sample (mg/L)
a	Gradient of the standard curve ($y = ax + b$)
b	Y-intercept of the standard curve ($y = ax + b$)
t	Time of the enzymatic reaction (5 minutes)
W	The weight of the tissue sample (0.1 g)
F_1	The dilution factor of the sample (if used; else = 1)
F_2	The dilution factor of the Total Amylase Test Solution (5×)