Alpha-Amylase Assay Kit

Catalog No.: abx097989

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 Alpha-Amylase activity in serum, saliva, and tissue homogenates.

Introduction

Alpha-Amylase is a key digestive enzyme, abundant in both the saliva and pancreatic juices, which assists in the breakdown of starch present in food. Like all amylases, Alpha-Amylase catalyzes the breaking of alpha-1,4-glycosidic bonds present in complex sugars, converting dietary starch into smaller disaccharides and trisaccharides. Alpha-Amylase is predominantly found in the digestive system of mammalian species, but is also synthesized by many plants, and some bacteria.

Abbexa's Alpha-Amylase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Alpha-Amylase activity. In the presence of starch, Alpha-Amylase catalyzes 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 Alpha-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.

- **Serum:** Serum samples can be tested directly.
- **Saliva:** Centrifuge at 10,000 × g at 4°C for 10 minutes. Take the supernatant, keep on ice, and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **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 × g for 10 minutes, then carefully remove the supernatant and dilute with distilled water for a final volume of 10 ml. Assay immediately, or aliquot and store at -80°C for up to 1 month.

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 cell lysates and 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, sample, and control. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 75 µl of sample to each 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 Substrate to the sample tubes.
5. Add 75 µl of Distilled water to the control tubes.
6. Incubate the samples and controls at 40°C for 5 minutes.
7. Add 75 µl of each standard dilution to the corresponding marked tubes.
8. Add 75 µl of Substrate to each standard tube.
9. Add 150 µl of Chromogenic Reagent to the samples, standards, and the control tubes.
10. 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 gently up and down to mix before adding to wells. Avoid foaming or bubbles.*
11. 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 Alpha-Amylase in each sample well can be derived with the formula:

1. Serum and Saliva samples:

One unit of Alpha-Amylase activity is defined as the amount required for 1 ml of serum or saliva to produce 1 mg of reducing sugar per minute.

$$\text{Alpha-Amylase (U/ml)} = \mathrm{F} \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}} - \mathrm{b}) \times \mathrm{V_{Substrate}}}{\mathrm{a} \times \mathrm{t} \times \mathrm{V_{Sample}}}$$

2. Tissue samples:

Alpha-Amylase activity in tissue samples can be calculated according to total protein concentration (which must be assayed separately) or according to sample weight.

Total Protein

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

$$\text{Alpha-Amylase (U/mg protein)} = \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}} - \mathrm{b}) \times \mathrm{V_{Substrate}}}{\mathrm{a} \times \mathrm{t} \times \mathrm{V_{Sample}} \times \mathrm{C_{Protein}}}$$

Sample Weight

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

$$\text{Alpha-Amylase (U/g tissue)} = \mathrm{F} \times \frac{\mathrm{V_{Tissue}}}{\mathrm{V_{Sample}}} \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}} - \mathrm{b}) \times \mathrm{V_{Substrate}}}{\mathrm{a} \times \mathrm{t} \times \mathrm{W}}$$

where:

OD_{Sample}	OD value of sample
$OD_{Control}$	OD value of control
$V_{Substrate}$	Volume of sample + Substrate (0.15 ml)
V_{Sample}	Volume of sample (0.075 ml)
V_{Tissue}	Volume of tissue preparation (10 ml)
$C_{Protein}$	Concentration of protein in sample (mg/ml)
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 mins)
W	The weight of the tissue sample (0.1 g)
F	The dilution factor of sample