Alpha-Amylase and Beta-Amylase Activity Assay Kit

Catalog No.: abx092188

Size: 96 tests

Detection Range: 0.01 U/ml – 0.37 U/ml

Sensitivity: 0.008 U/ml

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

Application: For detection and quantification of amylase activity in serum, plasma, tissue homogenates and saliva.

Introduction

Abbexa's Alpha-Amylase and Beta-Amylase Activity Assay Kit is a quick, convenient, and sensitive method for measuring and calculating amylase activity. Under heating conditions, reducing sugars generated by α-amylase and β-amylase react with 3,5-dinitrosalicylic acid (DNS) to produce a brown-red colored compound with an absorbance maximum at 540 nm. The intensity of the color is proportional to the amount of reducing sugars produced and can be used to calculate α-amylase and β-amylase activity.

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 (535 - 540 nm)
2. Double-distilled water
3. Pipette and pipette tips
4. Vials/tubes
5. Water bath
6. Centrifuge
7. Vortex mixer

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Protocol

A. Preparation of samples and reagents

1. Reagents

- **Standards:** Label 7 tubes with 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, 1.0 mg/ml, 1.2 mg/ml, and 1.4 mg/ml. Prepare these dilutions according to the volumes in the following table:

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

For the blank, use pure double-distilled water. The volume of each standard dilution 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 70-80°C and stir gently to dissolve the precipitate. Allow the tubes to cool back to 40°C before use.
- Generally, absorbance values of samples tested should be < 0.8. If higher values are observed, dilute the sample before testing.
- If there is any precipitation in the final test supernatant, before testing centrifuge at 4000 × g for 5 minutes.

2. 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 and plasma:** Serum and plasma samples can be tested directly.
- **Saliva:** Centrifuge samples at 3,000 × g for 10 minutes to remove particulate matter. Fresh samples are recommended.
- **Tissue Homogenates:** Carefully weigh 0.1 g of tissue and add it to 0.9 ml of double-distilled water. Homogenize manually or using a mechanical homogenizer in an ice-water bath. Transfer the homogenate to a clean tube and incubate at room temperature for 15 minutes, vortexing every 5 minutes during the incubation period. Centrifuge at 3,000 x g for 10 minutes at room temperature. Collect the supernatant and dilute with double-distilled water to a final volume of 10 ml. Mix thoroughly and assay immediately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before

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carrying out the formal experiment. Dilute samples into different concentrations with double-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
Human plasma	10 - 15
Human serum	10 - 15
Human saliva	15 - 25
Mouse serum	15 - 25
1% corn grain tissue homogenate	2 - 5

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.

B. Assay Procedure

1. Prepare and label standard, blank, sample, and control tubes. Each sample requires a corresponding control. It is strongly recommended that all measurements be performed in duplicate.
2. Add 75 µl of each standard dilutions to the corresponding standard tubes.
3. Add 75 µl of double-distilled water to the blank tubes.
4. Add 75 µl of sample to the sample tubes and the corresponding control tubes.
5. Add 75 µl of Substrate to the standard, blank and sample tubes.
6. Add 75 µl of double-distilled water to the control tubes.
7. Incubate the sample tubes and control tubes in a 40°C water bath for 5 minutes.
8. Add 150 µl of Chromogenic Reagent to all tubes.
9. Mix thoroughly and incubate all tubes at 95°C for 5 minutes.
10. Cool the tubes under running water and transfer 250 µl from each tube to a microplate well.
11. Measure the OD of each well with a microplate reader at 540 nm.

C. Calculation of Results

Average the duplicate readings for each standard dilution. Subtract the mean OD value of the blank (0 mg/ml) from all standard readings to obtain the corrected OD values. Plot the standard curve using the corrected OD value of the standard dilutions on the y-axis, and their corresponding concentrations on the x-axis. The curve should form a straight line described by the formula $y = ax + b$.

Based on this curve, the activity of Alpha-Amylase and Beta-Amylase in each sample well can be derived using the following formulae:

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1. Serum, plasma and saliva samples:

One unit of amylase activity is defined as the quantity of enzyme in 1 ml of sample that catalyzes the consumption of 1 mg of reducing sugar in one minute at 37°C.

$$(\alpha+\beta)\ \text{Amylase activity (U/ml)} = \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}} - \mathrm{b})}{\mathrm{a} \times \mathrm{t} \times \mathrm{V_2}} \times V_3 \times \mathrm{f}$$

2. Tissue homogenates:

a) Calculation according to protein concentration of sample:

One unit of amylase activity is defined as the quantity of enzyme in 1 mg of tissue protein that catalyzes the consumption of 1 mg of reducing sugar in one minute at 37°C.

$$(\alpha+\beta)\ \text{Amylase activity}\ (\frac{\mathrm{U}}{\text{mg protein}}) = \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}} - \mathrm{b})}{\mathrm{a} \times \mathrm{t} \times \mathrm{C_{Protein}} \times \mathrm{V_2}} \times \mathrm{V_3} \times \mathrm{f}$$

b) Calculation according to fresh weight of sample:

One unit of amylase activity is defined as the quantity of enzyme in 1 mg of tissue that catalyzes the consumption of 1 mg of reducing sugar in one minute at 37°C.

$$(\alpha+\beta)\ \text{Amylase activity}\ (\frac{\mathrm{U}}{\text{g fresh weight}}) = \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}} - \mathrm{b})}{\mathrm{a} \times \mathrm{t} \times \mathrm{W} \times \mathrm{V_2}} \times \mathrm{V_3} \times \mathrm{V_1} \times \mathrm{f}$$

where:

$\mathrm{OD_{Sample}}$	OD value of the sample.
$\mathrm{OD_{Control}}$	OD value of the corresponding control
$\mathrm{V_1}$	Volume of the tissue preparation (10 ml)
$\mathrm{V_2}$	Volume of sample added to the reaction (0.075 ml)
$\mathrm{V_3}$	Volume of the enzymatic reaction (sample volume + volume of substrate = 0.15 ml)
$\mathrm{C_{Protein}}$	Concentration of protein in sample (mg/ml)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
t	Time for the enzymatic reaction (5 minutes)
W	Weight of the tissue sample (0.1 g)
f	Dilution factor of the sample

Technical Support

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

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