Technical Manual

β-Amylase Activity Assay Kit

- **Catalogue Code: MAES0018**
- **Size: 96T**
- **Research Use Only**

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.97-34.74 U/g

Sensitivity:

0.97 U/g

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

β-Amylase is a non-metallic exo-amylase, which decomposes polyglucan at the α-1,4-glycosidic bond, releasing β-maltose and trace β-limit dextrin. β-Amylase is mainly distributed in higher plants and is also found in some microorganisms. In abiotic stress, the enzyme plays a key role in starch degradation, early seed germination and cell protection.

3. Intended Use

This kit can measure β-Amylase activity in plant tissue samples.

4. Detection Principle

The reducing sugar reacts with 3,5-dinitrosalicylic acid under heating conditions to produce a brown-red substance. β-amylase was inactivated by the property of amylase not to be heat-resistant, and then the enzyme activity of total amylase and α-amylase is determined. So the activity of β-amylase can be calculated indirectly.

5. Kit components & storage

Item	Specification	Storage
Substrate	10 mL×1 vial	2-8°C, 6 months
Chromogenic Agent	20 mL×1 vial	2-8°C, 6 months, avoid direct sunlight
Standard (10 mg/mL)	1.5 mL×1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- 95°C Water bath
- Centrifuge
- Microplate Reader (535-540 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

1. For measuring the OD value, if there is precipitation, centrifuge at 4000 g for 5 min at room temperature and take the supernatant for determination.

2. When the absolute OD value is greater than 0.747, sample should be diluted appropriately.

7. Reagent Preparation

1. Bring all reagents to room temperature before use. Before the experiment, preheat substrate and chromogenic agent at 40°C for 10 min.

2. If there is precipitation in substrate, please use it after heating and dissolving at 70°C.

3. If there is yellow precipitation in chromogenic agent, please use it after heating and dissolving at 70°C.

8. Sample Preparation

Tissue sample:

1. Weigh 0.1 g sample, add 0.9 mL of distilled water and homogenized with a homogenizer, then transfer to the EP tube, incubate at room temperature for 15 min and oscillate every 5 min. Centrifuge at 3000 g at room temperature for 10 min, take the supernatant and add double distilled water to the final volume of 10 mL, mix fully and it is the α-amylase solution.

2. Take 1 mL amylase solution and add 4 ml of distilled water, mix fully to prepare diluted amylase solution which is for the measurement of (α+β) amylase activity.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.97-34.74 U/g).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor
1% Epipremnum aureum tissue homogenate	1
1% Green pepper tissue homogenate	1
1% Corn grain tissue homogenate	1
1% Daucus carota tissue homogenate	1

Note: The diluent is double distilled water.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 540 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	α1'	α1	α5'	α5	α9'	α9	α13'	α13	α17'	α17
B	B	B	T1'	T1	T5'	T5	T9'	T9	T13'	T13	T17'	T17
C	C	C	α2'	α2	α6'	α6	α10'	α10	α14'	α14	α18'	α18
D	D	D	T2'	T2	T6'	T6	T10'	T10	T14'	T14	T18'	T18
E	E	E	α3'	α3	α7'	α7	α11'	α11	α15'	α15	α19'	α19
F	F	F	T3'	T3	T7'	T7	T11'	T11	T15'	T15	T19'	T19
G	G	G	α4'	α4	α8'	α8	α12'	α12	α16'	α16	α20'	α20
H	H	H	T4'	T4	T8'	T8	T12'	T12	T16'	T16	T20'	T20

Note: A-H, standard wells; α_1'-α_{20}', control wells of α-amylase; α_1-α_{20}, sample wells of α-amylase T_1'- T_{20}', control wells of (α+β) amylase; T_1-T_{20}, sample wells of (α+β) amylase

10. Operation Steps

The preparation of standard curve

Dilute standard (10 mg/mL) with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 mg/mL.

The measurement of standard

1. Take 1.5 mL EP tube and number the tubes from A to H in duplication, add 75 μL of standard solution with different concentrations to the corresponding tubes.

2. Add 75 μL of substrate to each tube.

3. Add 150 μL of chromogenic agent to each tube.

4. Mix fully and incubate at 95°C for 5 min. Cool the tubes with running water and take 250 μL of supernatant to the microplate. Measure the OD value of each well with microplate reader at 540 nm.

The measurement of α-amylase activity in sample (every sample tube need a control tube)

1. **Sample tube:** Add 75 μL of α-amylase solution to the corresponding tubes.
   Control tube: Add 75 μL of α-amylase solution to the corresponding tubes.

2. Incubate at 70°C water bath for 15 min and cool the tubes with running water.

3. **Sample tube:** Add 75 μL of substrate to the corresponding tubes.
   Control tube: Add 75 μL of double distilled water to the corresponding tubes.

4. Incubate the sample tubes and control tubes at 40°C water bath for 5 min.

5. Add 150 μL of chromogenic agent to each tube.

6. Mix fully and incubate at 95°C for 5 min. Cool the tubes with running water and take 250 μL of supernatant to the microplate. Measure the OD value of each well with microplate reader at 540 nm.

The measurement of (α+β) amylase activity in sample (every sample tube need a control tube)

1. **Sample tube:** Add 75 μL of diluted amylase solution to the corresponding tubes.
   Control tube: Add 75 μL of diluted amylase solution to the corresponding tubes.

2. **Sample tube:** Add 75 μL of substrate to the corresponding tubes.
   Control tube: Add 75 μL of double distilled water to the corresponding tubes.

3. Incubate the sample tubes and control tubes at 40°C water bath for 5 min.

4. Add 150 μL of chromogenic agent to each tube.

5. Mix fully and incubate at 95°C for 5 min. Cool the tubes with running water and take 250 μL of supernatant to the microplate. Measure the OD value of each well with microplate reader at 540 nm.

Operation Table

The measurement of standard

	Standard tubes
Standard solution with different concentrations (μL)	75
Substrate (μL)	75
Chromogenic agent (μL)	150
Mix fully and incubate at 95°C for 5 min. Cool the tubes with running water and take 250 μL of supernatant to the microplate. Measure the OD value of each well with microplate reader at 540 nm.	

The measurement of α-amylase

	Control tubes	Sample tubes
α-Amylase solution (μL)	75	75
Incubate at 70°C water bath for 15 min and cool the tubes with running water.		
Double distilled water (μL)	75	
Substrate (μL)		75
Incubate the sample tubes and control tubes at 40℃ water bath for 5 min.		
Chromogenic agent (μL)	150	150
Mix fully and incubate at 95°C for 5 min. Cool the tubes with running water and take 250 μL of supernatant to the microplate. Measure the OD value of each well with microplate reader at 540 nm.		

The measurement of (α+β) amylase

	Control tubes	Sample tubes
Diluted amylase solution (μL)	75	75
Double distilled water (μL)	75	
Substrate (μL)		75
Incubate the sample tubes and control tubes at 40°C water bath for 5 min.		
Chromogenic agent (μL)	150	150
Mix fully and incubate at 95°C for 5 min. Cool the tubes with running water and take 250 μL of supernatant to the microplate. Measure the OD value of each well with microplate reader at 540 nm.		

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Calculate according to the protein concentration of the sample

Definition: The production of 1 mg reducing sugar catalyzed by 1 mg of tissue protein per minute that is defined as an enzyme activity unit.

$$\text{α-amylase activity (U/mg prot)} = (\Delta A - b) \div a \times V_3 \div t \div V_2 \div C_{pr}$$

$$\text{(α+β) amylase activity (U/mgprot)} = (\Delta A - b) \div a \times V_3 \div t \div V_2 \div C_{pr} \times 5^*$$

2. Calculate according to the fresh weight of sample

Definition: The production of 1 mg reducing sugar catalyzed by 1 g of tissue per minute that is defined as an enzyme activity unit.

$$\text{α-amylase activity (U/g fresh weight)} = (\Delta A - b) \div a \times V_3 \div t \div w \times \frac{V_1}{V_2} \times f$$

$$\text{(α+β) amylase activity (U/g fresh weight)} = (\Delta A - b) \div a \times V_3 \div t \div w \times \frac{V_1}{V_2} \times f \times 5^*$$

$$(\alpha + \beta)\text{Amylase activity (U/g fresh weight)} = (\Delta A - b) \div a \times V_3 \div t \div w \times \frac{V_1}{V_2} \times f$$

β-amylase activity = (α+β) amylase activity - α-amylase activity

y: $OD_{Standard} - OD_{Blank}$. ($OD_{Blank}$ is the OD value when the standard concentration is 0)

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

f: Dilution factor of sample before tested.

ΔA: $OD_{Sample} - OD_{Control}$

V_1: The volume of prepared tissue sample in sample preparation step (10 mL).

V_2: The volume of sample added to the reaction (0.075 mL).

V_3: The volume of enzymatic reaction (the volume of sample + the volume of substrate = 0.15 mL).

t: The time of enzymatic reaction (5 min).

w: The weight of tissue sample (0.1 g).

C_{pr}: Concentration of protein in sample (mgprot/mL).

5*: Dilution factor for the preparation of diluted amylase solution.

12. Performance Characteristics

Detection Range	0.97-34.74 U/g
Sensitivity	0.97 U/g
Average recovery rate (%)	96
Average inter-assay CV (%)	3.2
Average intra-assay CV (%)	2.3

Analysis

Take 0.1 g of green pepper, treat the sample according to the sample preparation step, take 0.1 mL of α-amylase solution and add 0.4 mL of double distilled water, mix fully to prepare the diluted amylase solution, carry the assay according to the operation table.

The results are as follows:

y = 0.8729 x – 0.0112, the average OD value of the sample is 0.368, the average OD value of the control is 0.247, and the calculation result of α-amylase activity is:

α-amylase activity (U/g fresh weight)

= (0.368-0.247+0.0112) ÷ 0.8729 × 0.15 ÷ 5 ÷ 0.1 × 10 ÷ 0.075 = 6.06 U/g tissue

The calculation of (α+β) amylase activity: the average OD value of the sample is 0.205, the average OD value of the control is 0.154, and the calculation result is:

(α+β) amylase activity (U/g fresh weight)

= (0.205-0.154+0.0112) ÷ 0.8729 × 0.15 ÷ 5 ÷ 0.1 × 10 ÷ 0.075 × 5

= 14.25 U/g fresh weight

β-amylase activity (U/g fresh weight) = 14.25 - 6.06 = 8.19 U/g fresh weight

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

Email: info@assaygenie.com **Web:** www.assaygenie.com

Notes:

Email: info@assaygenie.com **Web:** www.assaygenie.com

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@assaygenie.com

Web: www.assayenie.com