TECHNICAL MANUAL

alpha-Galactosidase (alpha-Gal) Activity Assay Kit

- **SKU CODE:** MAES0432
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Operating steps
- Calculation

2. Intended use

This kit can be used to measure α-galactosidase (α-Gal) activity in animal and plant tissue samples.

3. Detection principle

α-Galactosidase (α-Gal) catalyzes the hydrolysis of substrates produced during the glycolysis process in animal and plant tissues to form the final product, which has a maximum absorption peak at 405 nm.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	50 mL × 2 vials	-20 °C, 12 months
Reagent 2	Substrate	Powder × 1 vial	-20 °C, 12 months, shading light
Reagent 3	Accelerant	1.6 mL × 1 vial	-20 °C, 12 months, shading light
Reagent 4	Chromogenic Agent	13 mL × 1 vial	-20 °C, 12 months
Reagent 5	10 mmol/L Standard Solution	0.3 mL × 1 vial	-20 °C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (395-415 nm, optimum wavelength: 405 nm)

Vortex mixer

Centrifuge

Reagents:

Double distilled water

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate working solution:** Dissolve one vial of substrate with 1.3 mL of double distilled water and mix well. Store at 2-8 °C for 4 weeks.
3. **Preparation of measuring working solution:** For each well, prepare 135 µL of measuring working solution by mixing 100 µL of buffer solution, 20 µL of substrate working solution, and 15 µL of accelerant. Mix well. Store at 2-8 °C for 1 month, protected from light.
4. **Preparation of control working solution:** For each well, prepare 135 µL of control working solution by mixing 120 µL of buffer solution and 15 µL of accelerant. Mix well. Store at 2-8 °C for 1 month, protected from light.
5. **Preparation of 0.5 mmol/L standard solution:** Dilute 0.05 mL of 10 mmol/L standard solution with 0.95 mL of double distilled water, and keep it on ice protected from light for detection. The 0.5 mmol/L standard solution should be prepared fresh and used within 8 hours.
6. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 0.5 mmol/L standard with double distilled water to create a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.15, 0.2, 0.3, 0.4, 0.45, 0.5 mmol/L.

7. Sample preparation

Sample preparation:

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL buffer solution with a dounce homogenizer at 4 °C.

4. Centrifuge at 10,000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

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

- 10% Sweet potato tissue homogenate: 1

- 10% Wood mushroom tissue homogenate: 1

- 10% Flammulina velutipes tissue homogenate: 1

- 10% Mouse liver tissue homogenate: 1

Note: The diluent is buffer solution. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. Operating steps

1. Standard well: Add 10 µL of standard solution with different concentrations to the corresponding wells. Sample well: Add 10 µL of sample to the corresponding wells. Control well: Add 10 µL of sample to the corresponding wells.
2. Add 135 µL of measuring working solution to the sample wells and standard wells. Add 135 µL of control working solution to the control wells.
3. Mix well with microplate reader for 5 seconds and incubate at 37 °C for 30 minutes. Add 100 µL of chromogenic agent to each well.
4. Mix well with microplate reader for 5 seconds, and measure the OD value of each well at 405 nm with the microplate reader.

9. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using the absolute OD value of standards and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

Tissue sample:

Definition: The amount of α-Gal in 1 g sample protein that hydrolyzes the substrate to produce 1 μmol product in 1 minute at 37 °C is defined as 1 unit.

α-Gal activity (U/g_{prot}) = (ΔA_405 - b) ÷ a ÷ T × f ÷ C_{pr} × 1000

[Note]

ΔA_405: OD_{Sample} – $OD_{control}$

f: Dilution factor of sample before test

C_{pr}: Concentration of protein in sample (g_{prot}/L)

T: Reaction time, 30 min

1000: 1 mmol/L = 1000 μmol/L

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three sweet potato tissue samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.50	16.00	27.00
%CV	3.0	2.6	1.0

Inter-assay Precision

Three sweet potato tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.50	16.00	27.00
%CV	8.0	6.9	4.0

Recovery

Three samples of high, middle, and low concentrations were tested 6 times in parallel to obtain an average recovery rate of 99%.

Recovery

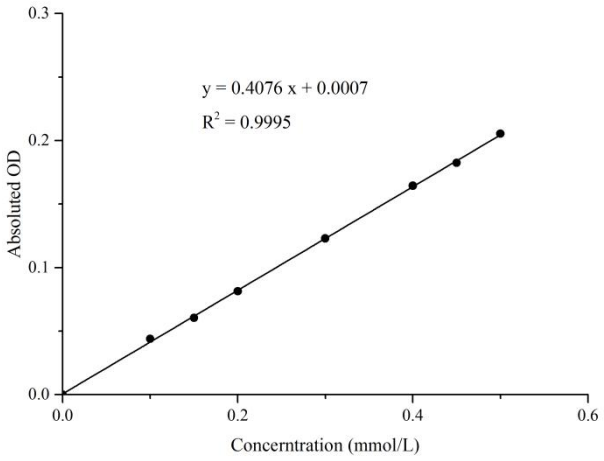
Sample	Expected Conc. (mmol/L)	Observed Conc. (mmol/L)	Recovery rate (%)
Sample 1	0.13	0.1	98
Sample 2	0.25	0.2	99
Sample 3	0.47	0.5	100

Sensitivity

The analytical sensitivity of the assay is 0.02 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve data

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (mmol/L)	OD value 1	OD value 2	Average OD	Absolute OD
0	0.1	0.1	0.1	0
0.1	0.144	0.143	0.144	0.044
0.15	0.161	0.159	0.160	0.061
0.2	0.181	0.181	0.181	0.081
0.3	0.221	0.224	0.223	0.123
0.4	0.264	0.264	0.264	0.164
0.45	0.281	0.283	0.282	0.182
0.5	0.305	0.305	0.305	0.205

11. Appendix II Example Analysis

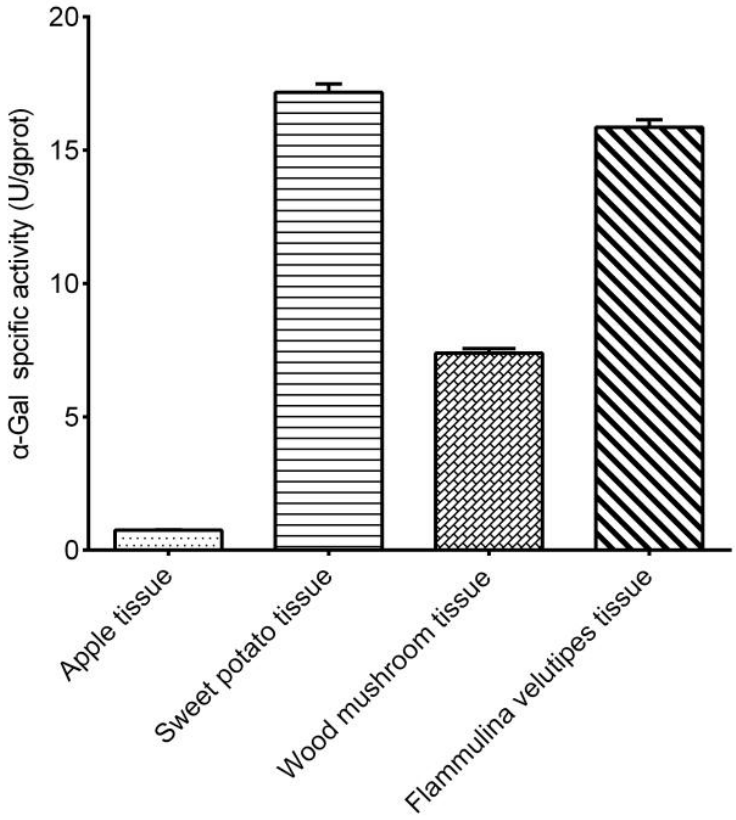
Example analysis:

Take 10 µL of 10% apple tissue supernatant sample and carry out the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.4076x + 0.0007, the average OD value of the control is 0.078, the average OD value of the sample is 0.130, the concentration of protein in sample is 5.63 g_{prot}/L, and the calculation result is:

α-Gal activity (U/g_{prot}) = (0.078 - 0.130 - 0.0007) ÷ 0.4076 ÷ 30 ÷ 5.630 × 100 = 0.75 U/g_{prot}

Detect 10% apple tissue homogenate (protein concentration: 5.63 g_{prot}/L), 10% sweet potato tissue homogenate (protein concentration: 3.60 g_{prot}/L), 10% wood mushroom tissue homogenate (protein concentration: 3.98 g_{prot}/L), and 10% Flammulina velutipes tissue homogenate (protein concentration: 2.23 g_{prot}/L) according to the protocol. The results are as follows:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use

of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.