TECHNICAL MANUAL

β-Glucosidase (β-GC) Activity Assay Kit

- **SKU CODE:** MAES0461
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate at 37°C for 30 min
- Measure OD values at 400 nm

2. Intended use

This kit can be used to detect β-glucosidase (β-GC) activity in tissue and fungal samples.

3. Detection principle

β-glucosidase (β-GC), also known as β-glucoside hydrolase, efficiently hydrolyzes terminal non-reducing β-D-glucoside bonds in various glycoside compounds, releasing β-D-glucose and corresponding ligands. This enzyme is widely present in nature, including plant seeds, animal intestine and other organs, and microorganisms.

β-GC is an important industrial enzyme with various applications. In medicine, β-GC catalyzes the degradation of glycoside groups to inhibit cancer cell expansion. In nutrition, β-GC catalyzes the production of anti-cancer isoflavones in soybeans. In bioenergy, β-GC is a key enzyme in converting cellulose to glucose.

β-GC catalyzes the reaction of substrate to produce chromogenic substances with maximum absorption at 400 nm. The enzyme activity is determined by measuring the optical density at 400 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months
Reagent 2	Buffer	25 mL × 1 vial	50 mL × 1 vial	-20°C, 12 months
Reagent 3	Substrate	2.5 mL × 1 vial	5 mL × 1 vial	-20°C, 12 months, protected from light

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 4	10 mmol/L Standard Solution	1 mL × 1 vial	2 mL × 1 vial	-20°C, 12 months, protected from light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of working solution:** For each well, prepare 180 µL of working solution by mixing 36 µL of substrate with 144 µL of buffer. The working solution should be protected from light and used within 1 day.
3. **Preparation of 200 µmol/L standard solution:** Before testing, prepare sufficient 200 µmol/L standard solution according to the number of test wells. For example, prepare 2500 µL of 200 µmol/L standard solution by mixing 50 µL of 10 mmol/L standard solution with 2450 µL of buffer. The 200 µmol/L standard solution should be prepared fresh and protected from light.
4. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 200 µmol/L standard solution with buffer to create a serial concentration. The recommended dilution gradient is: 0, 40, 60, 80, 120, 140, 160, 200 µmol/L.

6. Sample preparation

1. **Tissue and fungal sample preparation:** 1) Harvest the amount of tissue or fungus needed for each assay (initial recommendation: 20 mg). 2) Wash tissue or fungus in cold PBS (0.01 M, pH 7.4). 3) Homogenize 20 mg tissue or fungus in 180 µL extraction solution using a dounce homogenizer at 4°C. 4) Centrifuge at 10,000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep on ice for detection. 5) Determine the protein concentration of supernatant using appropriate protein assay kits.
2. **Sample dilution:** The recommended dilution factors for different samples are provided as reference: 10% Apple seeds homogenate: 50-60; 10% Spinach tissue homogenate: 1; 10% Shiitake tissue homogenate: 3-5; 10% Grapefruit seeds homogenate: 1; 10% Corn tissue homogenate: 1; 10% Mushroom tissue

homogenate: 1; 10% Cabbage tissue homogenate: 1; 10% Mouse small intestine tissue homogenate: 1; 10% Pear seeds homogenate: 2-4. Use extraction solution as diluent. For other sample types, perform a pretest to determine the appropriate dilution factor.

7. Operating steps

1. Standard wells: Add 200 µL of standard to the corresponding wells. Sample wells: Add 20 µL of sample to the corresponding wells.

2. Add 180 µL of working solution to sample wells.

3. Mix thoroughly with microplate reader for 5 seconds and measure the initial OD value (A1) of sample wells at 400 nm.

4. Incubate at 37°C for 30 minutes and measure the final OD value (A2) of each well at 400 nm.

8. Calculation

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 to obtain the absolute OD value.

3. Plot the standard curve using absolute OD values of standards as y-axis and corresponding concentrations as x-axis. Create the standard curve (y = ax + b) using graphing software or Excel.

Sample calculation:

Tissue and fungal samples:

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

β-GC activity (U/g_{prot}) = (ΔA_400 - b) ÷ a ÷ T × f ÷ C_{pr} × 10

Where:

ΔA_400: $\Delta A_400 = A_2 - A_1$

f: Dilution factor of sample before test

T: Reaction time, 30 min

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

10: Dilution factor when sample is added to the reaction system

9. Appendix

Performance Characteristics

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.50	2.50	3.20
%CV	2.8	3.2	4.5

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.50	2.50	3.20
%CV	2.0	9.7	5.6

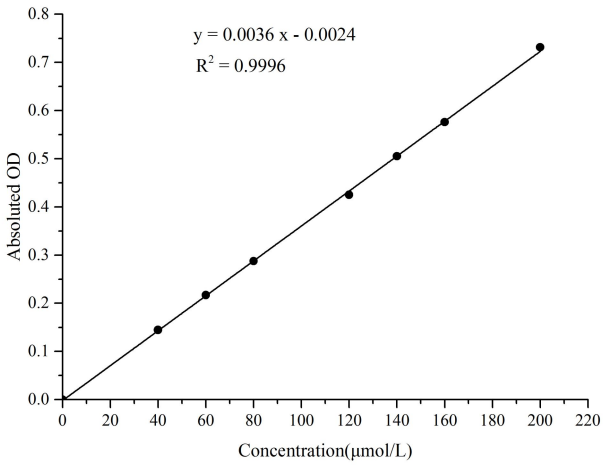
Recovery

Standard 1	Standard 2	Standard 3
50	100	150
49.5	102	156
99	102	104

Sensitivity

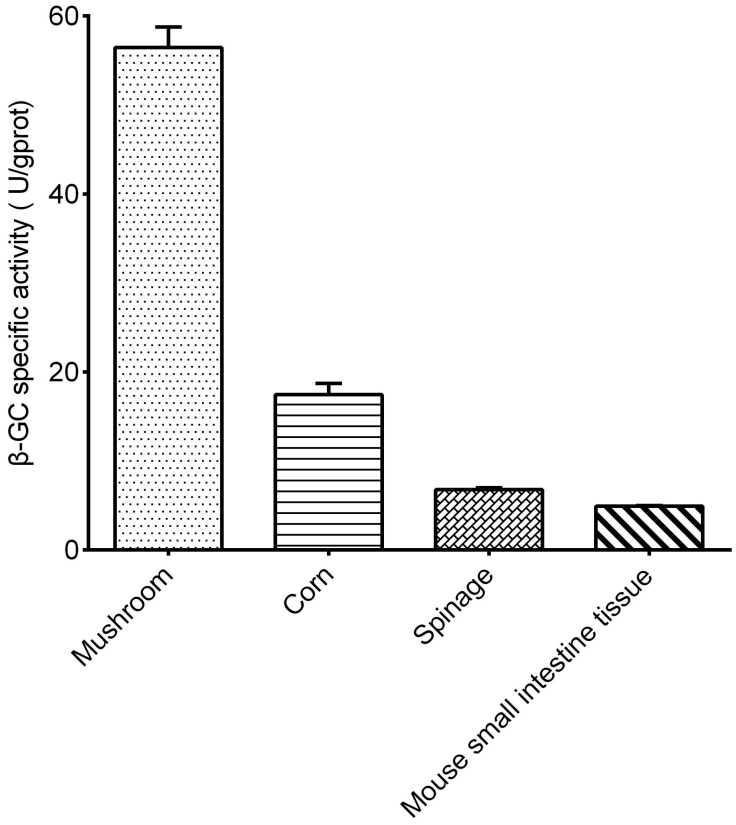
The analytical sensitivity of the assay is 0.06 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve data



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

Example Analysis



Example analysis:

Take 20 µL of 10% mouse small intestine tissue homogenate and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.0036x - 0.0019

Average A_1 of sample: 0.240

Average A_2 of sample: 0.644

$\Delta A_400 = A_2 - A_1 = 0.644 - 0.240 = 0.404$

Protein concentration in sample: 7.75 g_{prot}/L

Calculation result:

β-GC activity (U/g_{prot}) = (0.404 + 0.0019) ÷ 0.0036 ÷ 30 × 1 ÷ 7.75 × 10 = 4.85 U/g_{prot}

Detection of various tissue homogenates according to the protocol showed the following results: 10% mushroom tissue homogenate (protein concentration: 0.69 g_{prot}/L), 10% corn tissue homogenate (protein concentration: 1.24 g_{prot}/L), 10% spinach tissue homogenate (protein concentration: 1.10 g_{prot}/L), and 10% mouse small intestine tissue homogenate (protein concentration: 7.75 g_{prot}/L).

10. 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.