



TECHNICAL MANUAL

α -Glucosidase (α -GC) Activity Assay Kit

- **SKU CODE:** MAES0460
- **SIZE:** 48T (32 samples) / 96T(80 samples)
- **PRODUCT TYPE:** Assay Kit
- **RUO:** Research-Use-Only

1. Key Features

Detection Range: 0.35-33.43 U/L

Measuring Instrument: Microplate reader(390-405 nm)

2. Intended use

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

3. Detection principle

α -Glucosidase (α -GC), also known as α -D-glucoside hydrolase, is widely distributed in nature with extensive species diversity and varying properties, existing in virtually all organisms. It plays critical physiological roles in carbohydrate metabolism across animals, plants, and microorganisms. α -GC deficiency leads to severe glycogen metabolism disorders and excessive glycogen accumulation, causing Pompe disease (an autosomal recessive inherited glycogen storage disease, also known as glycogenosis type II). Chronic low α -GC activity in human tissues results in muscle fiber destruction and muscle atrophy.

This kit employs a detection principle whereby the chromogenic product generated from substrate hydrolysis catalyzed by α -glucosidase exhibits maximum absorption at 400 nm. The enzyme activity of α -GC is determined by measuring the optical density at 400 nm.

4. Kit components & storage

Store all reagents under the conditions specified in the table above. Reagents from different kit lots cannot be mixed. For small volume reagents, centrifuge briefly before use to ensure sufficient reagent recovery.

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 Solution	7 mL × 1 vial	14 mL × 1 vial	-20°C, 12 months
Reagent 3	Substrate	0.5 mL × 1 vial	1 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	10 mmol/L Standard Solution	1 mL × 1 vial	1 mL × 2 vials	-20°C, 12 months, shading light

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments

Microplate reader (390-405 nm, optimal wavelength: 400 nm), Incubator (37°C)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.

2. Working solution preparation:

For each well, prepare 120 μL of working solution by mixing 8 μL of substrate and 112 μL of buffer solution thoroughly. The working solution should be used within 1 day.

3. 1 mmol/L standard solution preparation:

Before testing, prepare sufficient 1 mmol/L standard solution according to the number of test wells. For example, prepare 1000 μL of 1 mmol/L standard solution by mixing 100 μL of 10 mmol/L standard solution with 900 μL of double-distilled water. The 1 mmol/L standard solution should be prepared fresh and protected from light.

4. Standard curve preparation:

Always prepare fresh standards. Discard working standard dilutions after use.

Dilute 1 mmol/L standard solution with double-distilled water to create serial concentrations. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.7, 1 mmol/L. Reference preparation is as follows:

Item	1	2	3	4	5	6	7	8
Concentration (mmol/L)	0	0.1	0.2	0.3	0.4	0.5	0.7	1
1 mmol/L Standard (μL)	0	20	40	60	80	100	140	200

Item	1	2	3	4	5	6	7	8
Double distilled water (µL)	200	180	160	140	120	100	60	0

7. Sample preparation

Tissues and fungus samples

1. Harvest the required amount of tissue or fungus 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 12,000×g for 15 min at 4°C to remove insoluble material. Collect the supernatant and keep on ice for detection.
5. Meanwhile, determine the protein concentration of the supernatant (MAES0177; MAES0126).

Dilution of sample

The recommended dilution factors for different samples are as follows (for reference only). For other sample types, perform a pretest to confirm the appropriate dilution factor.

Sample type	Dilution factor
10% Mouse small intestine tissue homogenization	1-2
10% Rat kidney tissue homogenization	1-2
10% Oranges seeds homogenization	1
10% Mouse kidney tissue homogenization	1-2
10% Apricot abalone mushroom tissue homogenization	1
10% Pear seeds homogenization	1
10% Corn tissue homogenization	1

Sample type	Dilution factor
10% Apple seeds homogenization	1

8. Operating steps

1. Standard wells: Add 20 μL of standard to the corresponding wells. Sample wells: Add 20 μL of sample to the corresponding wells.
2. Add 120 μL of buffer solution to standard wells. Add 120 μL of working solution to sample wells.
3. Mix thoroughly using the microplate reader for 5 s and measure the OD value (A1) of sample wells at 400 nm.
4. Incubate at 37°C for 30 min and measure the OD value (A2) of each well at 400 nm.

9. Calculation

The standard curve

1. Average the duplicate readings for each standard.
2. Subtract the mean OD (A2) value of the blank (Standard #1) from all standard readings. This gives the absolute OD value.
3. Plot the standard curve using the absolute OD values of standards and corresponding concentrations as y-axis and x-axis respectively. Create the

$$y = ax + b$$

standard curve ($y = ax + b$) using graphing software (or Excel).

Definition

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

$$\alpha\text{-GC activity} = (\Delta A_{400} - b) \div a \div T \times f \div C_{\text{pr}} \times 1000^* \text{ (U/g}_{\text{prot}})$$

[Note]

$$\Delta A_{400}: \Delta A_{400} = A_2 - A_1.$$

f: Dilution factor of sample before test.

t: Reaction time, 30 min.

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

1000*: 1 mmol/L = 1000 μ mol/L.

10. Appendix I Performance Characteristics

Intra-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.00	15.00	20.00
%CV	4.4	5.0	5.7

Inter-assay Precision

Three rat kidney samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.00	15.00	20.00
%CV	4.2	4.6	6.6

Recovery

Three samples of high, medium, and low concentrations were tested with 6 parallel replicates for each concentration to obtain an average recovery rate of 105%.

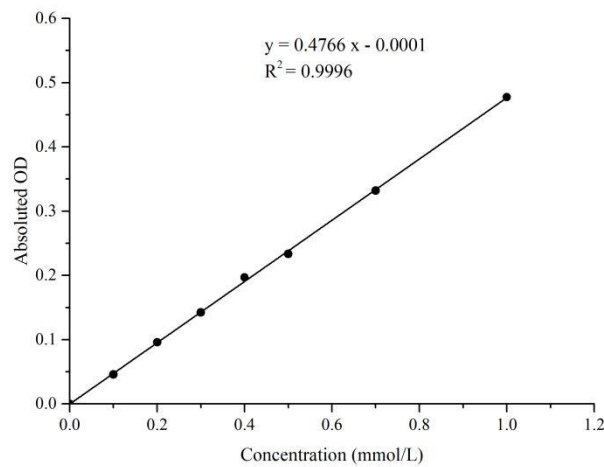
Standard 1	Standard 2	Standard 3
0.15	0.35	0.60
0.15	0.37	0.66
98	106	110

Sensitivity

The analytical sensitivity of the assay is 0.35 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.

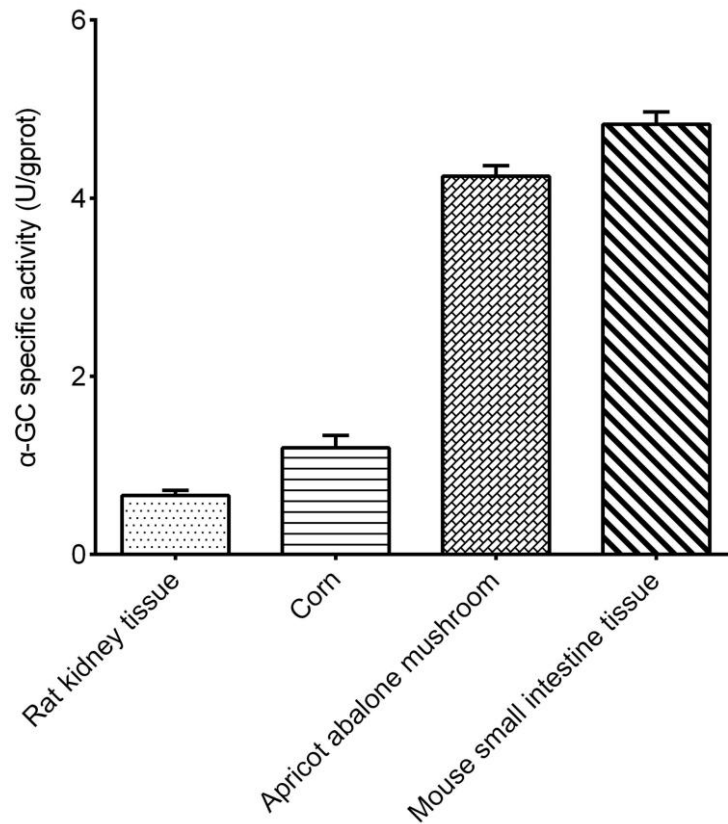
2. Standard curve

As 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 are provided below for reference only.



Concentration (mmol/L)	0	0.1	0.2	0.3	0.4	0.5	0.7	1.0
OD value	0.040	0.086	0.136	0.184	0.256	0.276	0.380	0.521
	0.041	0.087	0.137	0.182	0.219	0.272	0.365	0.515
Average OD	0.041	0.087	0.137	0.183	0.238	0.274	0.373	0.518
Absolutod OD	0	0.046	0.096	0.143	0.197	0.234	0.332	0.478

11. Appendix II Example Analysis



Example analysis

Using 10% mouse small intestine tissue homogenate diluted 2-fold, add 20 μL of diluted sample and perform the assay according to the operating steps. The results are as follows:

Standard curve: $y = 0.4766x - 0.0001$, the average A_1 of the sample is 0.095, the average A_2 of the sample is 0.407, $\Delta A_{400} = A_2 - A_1 = 0.407 - 0.095 = 0.312$, the protein concentration in the sample is 9.06 $\text{g}_{\text{prot}}/\text{L}$ and the calculation result is:

$$\alpha\text{-GC activity (U/g}_{\text{prot}}) = (0.312 + 0.0001) \div 0.4766 \div 30 \times 2 \div 9.06 \times 1000 = 4.82 \text{ U/g}_{\text{prot}}$$

Detection of 10% rat kidney tissue homogenate (protein concentration: 12.22 $\text{g}_{\text{prot}}/\text{L}$, diluted 2-fold), 10% corn tissue homogenate (protein concentration: 1.56 $\text{g}_{\text{prot}}/\text{L}$), 10% apricot abalone mushroom tissue homogenate (protein concentration: 0.34 $\text{g}_{\text{prot}}/\text{L}$), and 10% mouse small intestine tissue homogenate (protein concentration: 9.06 $\text{g}_{\text{prot}}/\text{L}$, diluted 2-fold) according to the protocol yielded the following results:

12. Statement

Assay Genie is not responsible for any problems or legal liabilities arising from using this kit for clinical diagnosis or other purposes.

2. Please read the instructions carefully and calibrate instruments before experiments. Follow the instructions strictly during experiments.
3. Protective measures must be taken by wearing laboratory coats and latex gloves.
4. If the substance concentration is not within the detection range, additional dilution or concentration should be performed on the sample.
5. A pretest is recommended if your sample is not listed in the instruction manual.
6. Experimental results are closely related to reagent conditions, operations, environment, and other factors. Assay Genie guarantees only the quality of the kits and is not responsible for sample consumption caused by using the assay kits. It is advisable to calculate possible sample usage and reserve sufficient samples before use.

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.