

**$\alpha$ -Glucosidase ( $\alpha$ -GC) Activity Assay Kit, Spectrophotometry****Product code:** 111971**Product Introduction**

$\alpha$ -Glucosidase ( $\alpha$ -GC, EC 3.2.1.20) is widely present in animals, plants, microorganisms, and cultured cells. It catalyzes the hydrolysis of  $\alpha$ -glycosidic bonds between aryl or hydrocarbon groups and glycosyl groups to produce glucose.  $\alpha$ -GC is associated with cell wall relaxation or strengthening, cell recognition, and the production of some signaling molecules.

In this assay,  $\alpha$ -GC decomposes p-nitrophenyl- $\alpha$ -D-glucopyranoside to produce p-nitrophenol, which has a maximum absorption peak at 400 nm.  $\alpha$ -GC activity is calculated by measuring the rate of increase in absorbance.

Actual readings may vary depending on detection conditions and instruments.

**Package Contents**

Code	Component	Size	Storage
111971.1	Reagent 1	2 × 60 mg	-20°C; protect from light
111971.2	Reagent 2	25 mL	2–8°C
111971.3	Reagent 3	50 mL	2–8°C
111971.4	Extraction Solution	50 mL	2–8°C
111971.m	Manual	1 copy	—

**Quality and Safety Information**

Material	Quality Standard	Main Toxicity
Reagent 1	—	—
Reagent 2	—	—
Reagent 3	—	—
Extraction Solution	—	—

**Transportation and Storage**

**Transportation:** This product is transported with ice packs.

**Storage:** Store each component according to the instructions above. Shelf life: 180 days.

**Instructions for Use****1. Preparation of Crude Enzyme Extract****Bacteria or Cultured Cells**

1. Collect bacteria or cells into a centrifuge tube, centrifuge, and discard the supernatant.
2. For every 4 million bacteria or cells, add 0.4 mL Extraction Solution.
3. Disrupt the bacteria or cells by ultrasonication in an ice bath: power 20% or 200 W, ultrasound 3 s, interval 10 s, repeat 30 times.
4. Centrifuge at 15000 × g, 4°C, for 20 min.
5. Collect the supernatant and keep it on ice for testing.

## Tissue

1. Add Extraction Solution according to the ratio of tissue mass (g) to Extraction Solution volume (mL) at 1:5–10. It is recommended to weigh approximately 0.1 g tissue and add 1 mL Extraction Solution.
2. Homogenize in an ice bath.
3. Centrifuge at  $15000 \times g$ ,  $4^{\circ}\text{C}$ , for 10 min.
4. Collect the supernatant and keep it on ice for testing.

## 2. Reagent Preparation

Before use, add 10 mL distilled water to each bottle of Reagent 1 and dissolve completely. Store any unused portion at  $-20^{\circ}\text{C}$ .

## 3. Assay Procedure

1. Preheat the spectrophotometer for more than 30 min.
2. Set the wavelength to 400 nm and zero the instrument with distilled water.
3. Add reagents and sample according to the table below.

Component	Control Tube	Assay Tube
Reagent 1	—	400 $\mu\text{L}$
Reagent 2	500 $\mu\text{L}$	500 $\mu\text{L}$
Sample	100 $\mu\text{L}$	100 $\mu\text{L}$

1. Mix thoroughly and incubate in a  $37^{\circ}\text{C}$  water bath for exactly 30 min.
2. Immediately place the tubes in a boiling water bath for 5 min. Tighten the caps to prevent water loss.
3. Cool under running water and mix thoroughly to ensure the concentration remains unchanged.
4. Add Reagent 1 to the control tube as shown below.

Component	Control Tube	Assay Tube
Reagent 1	400 $\mu\text{L}$	—

1. Mix thoroughly, centrifuge at  $8000 \times g$ ,  $4^{\circ}\text{C}$ , for 5 min, and collect the supernatant.
2. Prepare the color reaction as shown below.

Component	Control Tube	Assay Tube
Supernatant	500 $\mu\text{L}$	500 $\mu\text{L}$
Reagent 3	1000 $\mu\text{L}$	1000 $\mu\text{L}$

Mix thoroughly and let stand at room temperature for 2 min. Measure absorbance at 400 nm. Calculate  $\Delta A = A_{\text{assay}} - A_{\text{control}}$ . Each assay tube requires one control tube.

## Calculation of $\alpha$ -GC Activity

The regression equation measured under standard conditions is:

$$y = 0.00543x - 0.0027$$

Where  $x$  is the standard concentration (nmol/mL), and  $y$  is the absorbance value.

### 1. Calculation by Sample Protein Concentration

**Unit definition:** The production of 1 nmol p-nitrophenol per mg tissue protein is defined as one enzyme activity unit.

$$\alpha\text{-GC activity (nmol/min/mg protein)} = [(\Delta A + 0.0027) \div 0.00543 \times V_{\text{reaction total}}] \div (V_{\text{sample}} \times C_{\text{pr}}) \div T = 61.39 \times (\Delta A + 0.0027) \div C_{\text{pr}}$$

Protein concentration may be determined using a BCA Protein Assay Kit.

## 2. Calculation by Sample Fresh Weight

**Unit definition:** The production of 1 nmol p-nitrophenol per g tissue is defined as one enzyme activity unit.

$$\alpha\text{-GC activity (nmol/min/g fresh weight)} = [(\Delta A + 0.0027) \div 0.00543 \times V_{\text{reaction total}}] \div (W \times V_{\text{sample}} \div V_{\text{sample total}}) \div T = 61.39 \times (\Delta A + 0.0027) \div W$$

## 3. Calculation by Bacterial or Cell Density

**Unit definition:** The production of 1 nmol p-nitrophenol per 10,000 bacteria or cells is defined as one enzyme activity unit.

$$\alpha\text{-GC activity (nmol/min/10}^4\text{ cells)} = [(\Delta A + 0.0027) \div 0.00543 \times V_{\text{reaction total}}] \div (500 \times V_{\text{sample}} \div V_{\text{sample total}}) \div T = 0.123 \times (\Delta A + 0.0027)$$

### Formula Parameters

Parameter	Meaning	Value
$V_{\text{reaction total}}$	Total volume of the reaction system	1 mL
$V_{\text{sample}}$	Sample volume added to the reaction system	0.1 mL
$V_{\text{sample total}}$	Volume of Extraction Solution added	1 mL
Cpr	Sample protein concentration	mg/mL
W	Sample mass	g
500	Total number of cells or bacteria	$500 \times 10,000$
T	Reaction time	30 min

## Precautions

1. This 50T product can test 24 samples.
2. Before the formal assay, select 2–3 samples with relatively large expected differences for a preliminary assay.
3. Required instruments and supplies, prepared by the user: visible spectrophotometer, benchtop centrifuge, water bath, adjustable pipettes, 1 mL glass cuvettes, mortar, ice, and distilled water.
4. This product is for scientific research by professionals only. It must not be used for clinical diagnosis or treatment, must not be used for food or drugs, and must not be stored in ordinary residences.
5. For safety and health, wear a lab coat and disposable gloves during operation.