

α -Glucosidase (α -GC) Activity Assay Kit - Micro Method**Product Information****Product code:** 111972

α -Glucosidase (α -GC, EC 3.2.1.20) is widely present in animals, plants, microorganisms, and cultured cells. It catalyzes the hydrolysis of α -glycosidic bonds formed between aryl or alkyl groups and glycosyl groups to produce glucose. This activity is related to cell wall loosening or strengthening, cell recognition, and the production of certain signaling molecules.

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

Actual readings may vary under different testing conditions and instruments. Reference data are for guidance only.

Package Contents

| Product Code | Component | Quantity | Storage Temperature |
|--------------|---------------------|----------|-----------------------------|
| 111972.1 | Reagent 1 | 72 mg | -20°C, protected from light |
| 111972.2 | Reagent 2 | 15 mL | 2-8°C |
| 111972.3 | Reagent 3 | 15 mL | 2-8°C |
| 111972.4 | Extraction Solution | 100 mL | 2-8°C |
| 111972.m | Instruction Manual | 1 copy | - |

Quality and Safety Information

| Raw Material or Packaging Name | 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 indicated storage conditions. Shelf life is 180 days.

Instructions for Use**1. Preparation of Crude Enzyme Extract****1.1 Bacteria or Cultured Cells**

1. Collect bacteria or cells into a centrifuge tube, centrifuge, and discard the supernatant.
2. Use a bacterial or cell count of $500-1000 \times 10^4$ cells per 1 mL Extraction Solution. A recommended ratio is 500×10^4 bacteria or cells plus 1 mL Extraction Solution.
3. Disrupt the bacteria or cells by ultrasonication in an ice bath at 20% power or 200 W. Sonicate for 3 s, pause for 10 s, and repeat 30 times.

4. Centrifuge at 15000g and 4°C for 10 min.
5. Collect the supernatant and keep it on ice until testing.

1.2 Tissue

1. Use a tissue mass (g) to Extraction Solution volume (mL) ratio of 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 15000g and 4°C for 10 min.
4. Collect the supernatant and keep it on ice until testing.

2. Reagent Preparation

Before use, add 12 mL distilled water to Reagent I and dissolve thoroughly. Store any unused portion at -20°C.

3. Assay Procedure

1. Preheat the spectrophotometer or microplate reader for at least 30 min.
2. Set the wavelength to 400 nm and zero the instrument with distilled water.
3. Add reagents and samples according to the table below.

| Component | Control Tube | Assay Tube |
|------------|--------------|-------------|
| Reagent I | - | 120 μ L |
| Reagent II | 150 μ L | 150 μ L |
| Sample | 30 μ L | 30 μ L |

Mix thoroughly and incubate in a 37°C water bath for exactly 30 min. Immediately transfer to a 95°C water bath for 5 min. Cap tightly to prevent water loss. Cool under running water and mix thoroughly to ensure the concentration remains unchanged.

| Component | Control Tube | Assay Tube |
|-----------|--------------|------------|
| Reagent I | 120 μ L | - |

Centrifuge at 8000 \times g and 4°C for 5 min. Take the supernatant and perform the following operations in a tube or 96-well plate.

| Component | Control Tube | Assay Tube |
|-------------|--------------|-------------|
| Supernatant | 70 μ L | 70 μ L |
| Reagent III | 130 μ L | 130 μ L |

Mix thoroughly and let stand at room temperature for 2 min. Measure the absorbance at 400 nm as A and calculate $\Delta A = A_{\text{assay}} - A_{\text{control}}$. A control tube is required for each assay tube.

α -GC Activity Calculation

4.1 Calculation Using a Micro Quartz Cuvette

The regression equation measured under standard conditions is $y = 0.00585x - 0.0027$, where x is the standard concentration in nmol/mL and y is the absorbance value.

4.1.1 Calculation Based on Sample Protein Concentration

Unit definition: The amount of enzyme that produces 1 nmol p-nitrophenol per minute per mg of tissue protein is defined as one enzyme activity unit.

$$\alpha\text{-GC activity (nmol/min/mg, prot)} = [(\Delta A + 0.0027) \div 0.00585 \times V_{\text{total reaction}}] \div (V_{\text{sample}} \times C_{\text{pr}}) \div T = 56.98 \times (\Delta A + 0.0027) \div C_{\text{pr}}$$

4.1.2 Calculation Based on Sample Fresh Weight

Unit definition: The amount of enzyme that produces 1 nmol p-nitrophenol per minute per g of tissue is defined as one enzyme activity unit.

$$\alpha\text{-GC activity (nmol/min/g, fresh weight)} = [(\Delta A + 0.0027) \div 0.00585 \times V_{\text{total reaction}}] \div (W \times V_{\text{sample}} \div V_{\text{total sample}}) \div T = 56.98 \times (\Delta A + 0.0027) \div W$$

4.1.3 Calculation Based on Bacterial or Cell Density

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

$$\alpha\text{-GC activity (nmol/min/10}^4\text{cells)} = [(\Delta A + 0.0027) \div 0.00585 \times V_{\text{total reaction}}] \div (500 \times V_{\text{sample}} \div V_{\text{total sample}}) \div T = 0.114 \times (\Delta A + 0.0027)$$

4.2 Calculation Using a 96-Well Plate

The regression equation measured under standard conditions is $y = 0.0039x - 0.0027$, where x is the standard concentration in nmol/mL and y is the absorbance value.

4.2.1 Calculation Based on Sample Protein Concentration

Unit definition: The amount of enzyme that produces 1 nmol p-nitrophenol per minute per mg of tissue protein is defined as one enzyme activity unit.

$$\alpha\text{-GC activity (nmol/min/mg, prot)} = [(\Delta A + 0.0027) \div 0.0039 \times V_{\text{total reaction}}] \div (V_{\text{sample}} \times C_{\text{pr}}) \div T = 85.47 \times (\Delta A + 0.0027) \div C_{\text{pr}}$$

4.2.2 Calculation Based on Sample Fresh Weight

Unit definition: The amount of enzyme that produces 1 nmol p-nitrophenol per minute per g of tissue is defined as one enzyme activity unit.

$$\alpha\text{-GC activity (nmol/min/g, fresh weight)} = [(\Delta A + 0.0027) \div 0.0039 \times V_{\text{total reaction}}] \div (W \times V_{\text{sample}} \div V_{\text{total sample}}) \div T = 85.47 \times (\Delta A + 0.0027) \div W$$

4.2.3 Calculation Based on Bacterial or Cell Density

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

$$\alpha\text{-GC activity (nmol/min/10}^4\text{cells)} = [(\Delta A + 0.0027) \div 0.0039 \times V_{\text{total reaction}}] \div (500 \times V_{\text{sample}} \div V_{\text{total sample}}) \div T = 0.171 \times (\Delta A + 0.0027)$$

Formula Parameters

| Parameter | Definition |
|-----------------------------|--|
| $V_{\text{total reaction}}$ | Total volume of the reaction system, 0.3 mL |
| V_{sample} | Sample volume added to the reaction system, 0.03 mL |
| $V_{\text{total sample}}$ | Volume of Extraction Solution added, 1 mL |
| C_{pr} | Sample protein concentration, mg/mL |
| W | Sample mass, g |
| 500 | Total number of cells or bacteria, 500×10^4 |
| T | Reaction time, 30 min |

Precautions

- This 100T kit can test 48 samples.
- Before formal measurement, use 2-3 samples with relatively large expected differences for preliminary testing.

- Required instruments and supplies are not included: microplate reader or spectrophotometer, benchtop centrifuge, water bath, adjustable pipette, 96-well plate or 1 mL cuvette, mortar, ice, and distilled water.
- This product is for scientific research by professionals only. It must not be used for clinical diagnosis or treatment, must not be used in food or drugs, and must not be stored in ordinary residences.
- For your safety and health, wear a lab coat and disposable gloves during operation.