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 × 10^4cells per 1 mL Extraction Solution. A recommended ratio is 500 × 10^4bacteria 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 μL
Reagent II	150 μL	150 μL
Sample	30 μL	30 μL

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 μL	-

Centrifuge at 8000 × 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 μL	70 μL
Reagent III	130 μL	130 μL

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

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

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.

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

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.

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

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.

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

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.

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

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.

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

Formula Parameters

Parameter	Definition
$V_{total\ reaction}$	Total volume of the reaction system, 0.3 mL
V_{sample}	Sample volume added to the reaction system, 0.03 mL
$V_{total\ sample}$	Volume of Extraction Solution added, 1 mL
Cpr	Sample protein concentration, mg/mL
W	Sample mass, g
500	Total number of cells or bacteria, 500×10^4
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.