Ver.251201

α-Glucosidase (*α* -GC) Activity Assay Kit

BC12012-01 (50T/24S)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Maltase, also known as α-glucosidase is widely present in animals, plants, microorganisms, and cultured cells. It catalyzes the hydrolysis of the α-glycosidic bond between an aryl or hydrocarbon group and a sugar moiety to produce glucose. This process is not only related to the relaxation or reinforcement of the cell wall but is also closely associated with cell recognition and the production of certain signaling molecules.

The decomposition of α-GC on p-nitrophenyl-α-D-pyranoglucoside generates p-nitrophenol, which has a maximum absorption peak at 400 nm. The activity of α-GC is calculated by measuring the rate of increase in absorbance.

Kit components

Reagent	Volume	Storage
Extract solution	50mL	2-8°C
Reagent I	Powder x 2	-20°C
Reagent II	25mL	2-8°C
Reagent III	80mL	2-8°C
Standard	1mL	2-8°C

Solution preparation:

1. **Reagent I**: Take one bottle and add 10 mL distilled water before use, fully dissolved. Unused reagents can be dispensed and stored at -20°C for 4 weeks. Avoid repeating freeze thaw cycles.

2. **Standard:** 5 μmol/mL p-nitrophenol solution.

Reagents and Equipment Required but Not Provided

Spectrophotometer, low temperature centrifuge, water bath/constant temperature incubator, adjustable pipette, mortar/homogenizer/cell ultrasonic crusher, 1 mL glass cuvette, ice and distilled water.

Protocol

1. Sample Extraction: (The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

I. **Bacteria or cells:** Collecting bacteria or cells into a centrifuge tube, discard supernatant after centrifugation. Suggest 10 million with 1 mL of Extract solution. Use ultrasonication to split bacteria or cells (power 200W, work time 3s, interval 10s, repeat for 30 times). Centrifuge at 15000g at 4°C for 20 min, take the supernatant and place it on ice for testing.

II. **Tissue sample:** Mass about 0.2g of sample tissue with 1 mL of Extract solution. Fully grind on ice, centrifuge at 15000g at 4°C for 20 min. Take the supernatant and place it on ice for testing.

III. **Liquid sample:** Detect sample directly. If the solution is turbid, the supernatant should be centrifuged for determination

2. Determination procedure:

1. Preheat the spectrophotometer for 30 min, adjust wavelength to 400 nm, set zero with distilled water.
2. Standard solution dilution: dilute 5 μmol/mL p-nitrophenol solution with distilled water to 100, 50, 25, 12.5, 6.25, 0 (Blank tube) nmol/mL.
3. The standard solution dilution can refer to the following table:

Number	Pre dilution concentration(nmol/mL)	Standard liquid volume (μL)	Distilled water volume (μL)	Diluted concentration (nmol/mL)
1	5000	100	400	1000
2	1000	200	1800	100
3	100	1000	1000	50
4	50	1000	1000	25
5	25	1000	1000	12.5
6	12.5	1000	1000	6.25
7	6.25	0	1000	0 (Blank tube)

Note: Each standard tube in the following experiment requires 500 μL of standard solution (be careful not to directly test the absorbance of the standard solution in this step).

4. Operation table:

Reagent name (μL)	Test tube (T)	Control tube (C)	Standard tube (S)
Reagent I	400	-	-
Reagent II	500	500	-
Sample	100	100	-
Mix well, 37°C water bath/constant temperature incubator for 30 minutes and then put it into boiling water bath for 5 min immediately (wrap the sealing film to prevent bursting), mixed thoroughly after cooling with running water (ensure the same concentration).			
Reagent I	-	400	-
Mix well, 8000 g, 4°C, centrifuge for 5 min, and take the supernatant for testing			
Supernatant	500	500	-
Standard	-	-	500
Reagent III	1000	1000	1000

Mix well, place it at room temperature for 2 minutes, detect the absorbance at 400 nm. Note the absorption values of test tube, control tube, blank tube and standard tube as AT, Ac, A_B and As, respectively.

Calculate = ΔA_T - Ac, Δ As=As – A_B. Each test tube needs one control tube. Standard curve and blank tube only need to be measured once or twice.

Calculations

I.Standard curve Establish a standard curve based on the concentration (x, nmol/mL) and absorbance (y, ΔAs) of the standard tube. According to the standard curve, ΔA (y, ΔA_T) is brought into the formula to calculate the sample product concentration x (nmol/mL).

II. α -GC activity calculation

a) Calculated by protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the reaction of 1 nmol of p-nitrophenol in 1 mL reaction system per hour every milligram protein.

α -GC activity (U/mg prot) = x ×Vrv ÷ (Cpr×Vs) +T= 20 × x ÷ Cpr

b) Calculated by sample mass

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the reaction of lnmol of p-nitrophenol in 1 mL reaction system per hour every gram sample.

α -GC activity (U/g mass) = x ×Vrv ÷(W×Vs ÷Ve) ÷ T= 20×x÷W

c) Calculated by bacteria or cell amount**:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the reaction of lnmol of p-nitrophenol in 1 mL reaction system per hour every 10^4 bacteria or cells.

α-GC activity (U/10^4 cell) = (x ×Vrv) ÷(1000×Vs ÷Ve) ÷ T= 0.02 × x

d) Calculated by liquid volume

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the reaction of lnmol of p-nitrophenol in 1 mL reaction system per hour every milliliter sample.

α -GC activity (U/mL) = (x ×Vrv) ÷Vs ÷ T= 20 × x

Vrv : Total reaction volume, 1 mL
Vs : Sample volume in reaction system, 0.1 mL
Cpr : Supernatant protein concentration, mg/mL
Ve : Extract solution volume,1 mL
W : Sample mass, g
1000: Bacteria or cell amount, 1 000x 10^4
T : Reaction time, 0.5 h.