Alpha-glucosidase Assay Kit

Cat #: orb1500029 (manual)

Trace method

Size: 100T /48S

Product composition and storage conditions :

No.	Specifications	Storage conditions
Extraction solution -ES02	Liquid 100mL×1 bottle	Store at 4°C
orb1500029-A	Powde×2 bottle	Store at -20°C Before use, add 6mL of distilled water into each bottle, dissolve it fully; the remaining reagent can be stored at -20°C for 4 weeks, avoid repeated freezing and thawing.
orb1500029-B	Liquid 15mL×1 bottle	Store at 4°C
orb1500029-C	Liquid 15mL×1 bottle	Store at 4°C
orb1500029-Standard (5 μmol/mL)	Liquid 1mL×1 tube	Store at 4°C

Introduction

Significance: A-GC, EC 3.2.1.20, is widely found in animals, plants, microorganisms, and cultured cells, catalyzes the hydrolysis of alpha-glycosidic bonds between aryl or hydrocarbon groups and glycosyl to produce glucose, which is not only related to the relaxation or reinforcement of cell walls, but also closely related to cell recognition and the production of some signaling molecules.

Principle:A-GC decomposes p-nitrobenzene -α-D glucopyranoside to form p-nitrophenol, which has a maximum absorption peak at 400 nm, and the α-GC activity is calculated by measuring the rate of increase in absorbance.

Own suppliers: Visible spectrophotometer/plate reader, ccryogenic centrifuge, water bath, adjustable pipette, micro quartz cuvette/96-wells plate, mortar/homogenizer, ice and distilled water.

Extraction of crude enzyme solution:

1. Bacteria or culture cells: collect bacteria or cells into the centrifuge tube, centrifuge and discard the supernatant; According to the number of bacteria or cells (10^4), extraction solution ES02 volume (mL) is 500 ~ 1000:1 ratio (5 million bacteria or cells are recommended to add 1 mL extraction ES02) , the bacteria or cells were broken by ultrasonic wave (ice bath, power 20% or 200 W, ultrasonic 3 s, interval 10 s, repeat 30 times) ; Then centrifuge for 10 min at 15000 g, 4℃, and take the supernatant and place it on ice for testing.

2. Tissue： According to the ratio of tissue mass (g) : ES02 volume (mL) : 1:5 ~ 10(about 0.1 g tissue should be taken and 1 mL ES02 should be added) , homogenized in ice bath, centrifuged at 15000 g 4C for 10 min, the supernatant was taken and put it on ice for testing.

Measurement operation:

1. Preheat the visible spectrophotometer or plate reader for at least 30 minutes, adjust the wavelength to 400 nm, and set it to zero with the distilled water.

2. Dilution of standard solution: 5 μmol/mL standard solution was diluted to 200,150,50,25,12.5,0 nmol/mL standard solution with distilled water before use.

3. Samples testing (add the following reagents in turn):

Reagent name	Measuring tube (ul)	Control tube (ul)	Standard tube (ul)
orb1500029-A	100		
orb1500029-B	150	150	
Sample	30	30	
Mix fully, put into 37℃ water bath for 30min, then immediately put into 95℃ water bath for 5min (cover tightly, to prevent water loss) , cooling with flow water and then mix fully (to ensure constant concentration)			
orb1500029-A		30	

Mix fully, then centrifuge at 8000g, 4℃ for 5min, take supernatant			
Supernatant	70	70	
Standard			70
orb1500029-C	130	130	130
Mix fully, stay at room temperature for 2min, the absorption value was determined at 400 nm, and record it as A-measuring, A-control, A-standard and A- blank respectively. Calculated ΔA-measuring = A-measure - A-control, ΔA-standard = A-standard - a-blank. A control tube is required for each measuring tube. Standard curve and blank tube only need to test 1-2 times.			

α-GC activity calculations:

The standard curve was established according to the concentration of the standard tube (x, nmol/mL), and the absorbance (y, ΔA-standard). The sample product concentration x (nmol/mL), was calculated by substituting ΔA (y, ΔA-measuring) into the formula according to the standard curve.

(1) Calculated by protein concentration of sample:

Definition of Units: Production of 1nmol p-nitrophenol per hour per mg of tissue protein is defined as a unit of enzyme activity.

α-GC activity (U/mg prot) = (x×V1) ÷ (V2×Cpr) ÷T=18.67x÷Cpr

Protein concentration quantification is recommended using our BCA Protein Assay Kit (orb1085948).

(2) Calculated by sample fresh weight:

Definition of Units: Production of 1nmol p-nitrophenol per hour per g tissues is defined as an enzyme activity unit.

α-GC activity (U/g fresh weight) = (x×V1) ÷ (W×V2÷V3) ÷T=18.67x÷W

(3) Calculated by number of bacteria or cells:

Definition of Units: Production of 1nmol p-nitrophenol per hour per 10,000 bacteria or cells is defined as an enzyme activity unit.

α-GC activity (U/10^4 cell) = (x×V1) ÷ (500×V2÷V3) ÷T=0.0374x

Note: Cpr：protein concentration of sample, mg/mL, protein concentration needs to be determined separately; V1：Total volume of reaction, 0.28mL； V2：Sample volume added in reaction, 0.03mL； V3：Volume of extraction solution added, 1mL； W：Sample mass, g；500：number of cells or bacteria, 5 million; T: Reaction time, 0.5h.

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