α-Glucosidase (α-GC) Assay Kit

Note: The reagents of this product are subject to change. Please note and strictly follow this instruction.

Detection instrument: Spectrophotometer/ Microplate reader

Cat No: BC2555

Size: 100T/48S

Components:

Extraction: Liquid 100 mL×1.Store at 2-8℃.

Reagent I: Powder×2. Store at -20℃. Add 6 mL distilled water to each bottle before use, fully dissolved. Unused reagents can be dispensed and stored at -20℃ for 4 weeks. Avoid repeating freeze thaw cycles.

Reagent II: Liquid 15 mL×1. Store at 2-8℃.

Reagent III: Liquid 15 mL×1. Store at 2-8℃.

Standard: Liquid 1 mL×1. Store at 2-8℃. 5 μmol/mL p-nitrophenol solution.

Product Description:

α-GC (EC 3.2.1.20) is widely existed in animals, plants, microorganisms and cultured cells, which catalyzes the hydrolysis of α-glycosidic bonds between aryl or hydrocarbyl groups and glycosyl groups to form 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.

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

Reagents and Equipment Required but Not Provided：

Spectrophotometer/microplate reader, desk centrifuge, water bath/constant temperature incubator, sonicator, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ice and distilled water.

Procedure:

I. Sample Extraction:

1. Bacteria or cells: collecting bacteria or cells into a centrifuge tube, discard supernatant after centrifugation. Suggest 10 million with 1 mL of Extraction. Use ultrasonication to split bacteria or cells (power 200W, work time 3s，interval 10s，repeat for 30 times). Centrifuge at 15000 g and 4℃ for 20 min. Supernatant is placed on ice for test.
2. Tissue sample: suggested 0.2 g of tissue with 1 mL of Extraction. Fully grind on ice, centrifuge at 15000 g and 4℃ for 20 min. Supernatant is placed on ice for test.
3. Liquid sample: detect sample directly. If the solution is turbid, the supernatant should be centrifuged for determination.

II. Determination procedure:

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1. Preheat the spectrophotometer/microplate reader 30 min, adjust wavelength to 400 nm, set spectrophotometer counter to zero with distilled water.

2.Standard working solution: dilute 5 μmol/mL p-nitrophenol solution with distilled water to 100, 50, 25, 12.5, 6.25, 0 (Blank tube) nmol/mL.

3.Add reagents with the following list:

Reagent name (μL)	Test tube	Control tube	Standard tube
Reagent I	100		
Reagent II	150	150	
Sample	30	30	
Mix well, 37℃ water bath/constant temperature incubator for 30 min and then put it into boiling water bath for 5 min immediately (cover tightly to prevent water loss), mixed thoroughly after cooling with running water (to ensure the same concentration).			
Reagent I		100	
Mix well, 8000 g, 4℃, centrifuge for 5 min, and take the supernatant (add the following reagents to the EP tube or 96 well flat-bottom plate)			
Supernatant	70	70	
Standard			70
Reagent III	130	130	130

Mix well, plac at room temperature for 2 minutes, detect the absorbance at 400 nm. Note the light absorption values of test tube, control tube, blank tube and standard tube as A_T, A_C, A_B and A_S, respectively and calculate $\Delta A_T=A_T - A_C$, $\Delta A_S=A_S - A_B$. Each test tube needs one control tube. Standard curve and blank tube only need to be measured once or twice.

III. Calculations：

1. Create standard curve

Establish a standard curve based on the concentration (x, nmol/mL) and absorbance (y, ΔAs) of the standard tube. Based on the standard curve, ΔA (y, ΔAt) was brought into the formula to calculate the sample product concentration x (nmol/mL).

2. α-GC activity calculation

1) Calculated by protein concentration:

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

α-GC Activity (U/mg prot) $=x\times V_1\div(Cpr\times V_2)\div T= 18.67\times x\div Cpr$

2) Calculated by sample weight

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

α-GC Activity (U/g fresh weight) $=x\times V_1\div(W\times V_2\div V_3)\div T= 18.67\times x\div W$

3) Calculated by bacteria or cell amount:

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

α-GC Activity (U/10^4 cell) =$(x \times V_1) \div (1000 \times V_2 \div V_3) \div T = 0.0187 \times x$

V_1: Total reaction volume, 0.28 mL;

V_2: Sample volume in reaction system, 0.03 mL;

Cpr: Supernatant protein concentration, mg/mL;

V_3: Extraction volume,1 mL;

W: Sample weight, g;

1000: Bacteria or cell amount, 1000×10^4;

T: Reaction time, 0.5 h.

Note:

Extraction contains ingredients that denature proteins, and protein content needs additional measurement if α-GC activity would be calculated by protein concentration.

References：

[1] Wang S Y, Camp M J, Ehlenfeldt M K. Antioxidant capacity and α-glucosidase inhibitory activity in peel and flesh of blueberry (Vaccinium spp.) cultivars[J]. Food Chemistry, 2012, 132(4): 1759-1768.

Related Products：

BC0340/BC0345	Glucogen Content Assay Kit
BC0360/BC0365	β-1,3-glucanase(β-1,3-GA) Activity Assay Kit
BC2510/BC2515	Trehalase Activity Assay Kit