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α-Glucosidase (α-GC) Assay Kit

Note: Take two or three different samples for prediction before test.

Detection instrument: Spectrophotometer

Cat No: AK0209

Size: 50T/24S

Components:

Extraction: 50 mL×1.Store at 4℃ .

Reagent I: Powder×2. Store at -20℃ . Add 10 mL distilled water to each bottle before use, fully dissolved. The rest reagent is still stored at -20°C.

Reagent Ⅱ: 25 mL×1. Store at 4℃ .

Reagent Ⅲ: 80 mL×1. Store at 4℃ .

Standard: liquid ×1. Store at 4℃ . 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.

Required material

Desk centrifuge, spectrophotometer, mortar/homogenizer, 1 mL glass cuvette, transferpettor, 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 20%, work time 3s，interval 10s，repeat for 30 times). Centrifuge at 15000g at 4℃ for 10 min. Supernatant is placed on ice for test.

2. Tissue sample:

Suggested 0.2g tissue with 1 mL of Extraction. Fully grind on ice, centrifuge at 15000g at 4℃ for 20 min. Supernatant is placed on ice for test.

II. Determination procedure:

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

2 Standard working solution:

Add 100 μL standard to 400 μL regent Ⅲ to form 1 μmol/mL p-nitrophenol solution, ten fold dilution to 100 nmol/mL, diluted with distilled water to 100, 50, 25, 12.5, 6.25, 0 nmol/mL.

3 Add reagents with the following list:

Reagent name (μL)	Test control (T)	Control tube (C)	Standard tube (S)
Reagent I	400		
Reagent Ⅱ	500	500	
Sample	100	100	
Mix well, 37℃ water bath 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		400	
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	500	500	
Standard			500
Reagent Ⅲ	1000	1000	1000

Mix well, plac at room temperature for 2 minutes, detect the absorbance at 400 nm and calculate $\Delta A=A_T-Ac$. Each test tube needs one control tube。

III. Calculation:

1 Create standard curve

A standard curve is established based on the concentration (y) of the standard tube and the absorbance (x).

2 According to the standard curve, calculate the sample concentration (nmol/mL) by taking ΔA(x) into the formula.

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 mg protein.

GC (U/mg prot) $=y\times V_1\div(Cpr\times V_2)\div T=20\times y\div Cpr$

Need additional measurement, it is recommended to use our BCA protein content assay kit.

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 g sample.

GC (U/g fresh weight) $=y\times V_1\div(W\times V_2\div V_3)\div T=20\times y\div W$

3) Calculated by bacteria or cell amount:

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.

GC ($U/10^4$ cell) $=(y\times V_1)\div(1000\times V_2\div V_3)\div T=0.02\times y$。

V_1 : Total reaction volume, 1 mL;

V_2 : Sample volume in reaction system, 0.1 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.

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:

AK0291/AK0290 Glucogen Content Assay Kit
AK0556/AK0555 β- 1,3-glucanase(β- 1,3-GA) Activity Assay Kit
AK0218/AK0217 Trehalase Activity Assay Kit