β-glucosidase (β-GC) Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: NA0686

Size:50T/24S

Components:

Extract solution: Liquid 50 mL×1. Storage at 4℃.

Solution I: Powder×2. Storage at -20℃. Add 10 mL of distilled water to per bottle before use and dissolve it fully. The left reagent store at -20℃.

Solution II: Liquid 25 mL×1. Storage at 4℃.

Solution III: Liquid 80 mL×1. Storage at 4℃.

Standard: Liquid 1 mL×1. Storage at 4℃.5 μmol/mL p-nitrophenol solution.

Product Description

β-glucosidase (β-GC, EC 3.2.1.21) is an enzyme found broadly in animals, plants, microorganisms and cultured cells, which catalyzes the hydrolysis of base-glycosidic bonds and has many physiological functions. In cellulose saccharification, β-GC is responsible for further hydrolysis of cellulose disaccharides and cellulose oligosaccharides to produce glucose. β-GC hydrolyzes the aroma precursors of terpenes, making the glycoside turns from bond state to free state, thus producing the fragrance. β-GC can also hydrolyze wild sakuraside in plants and release HCN, thereby preventing insects from eating.

β-GC can catalyze the p-nitrophenyl-β-D-glucopyranoside to p-nitrophenol. The product has characteristic of absorption at 400 nm. In this kit, the β-GC activity is quantified by measuring the increase in the color development at 400 nm.

Reagents and Equipment Required but Not Provided.

Centrifuge, water-bath, transferpettor, spectrophotometer, 1 mL glass cuvette, ice, mortar/homogenizer and distilled water.

Procedure

I. Preparation of standard samples:

1. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. According to the number of bacteria or cells (10^4): the volume of the Extract solution (mL) is 500-1000:1. Suggest add 1 mL of Extract solution to 10 million of bacteria or cells. Use ultrasonication to splitting bacteria and cells (placed on ice, ultrasonic power 20% or 200W, working time 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 15000×g for 20 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

2. Tissue

Add 1 mL of Extraction reagent to 0.2 g of tissue, and fully homogenized on ice bath. According to the number of sample weight (g): the volume of the Extract solution (mL) is 1:5-10. Centrifuge at 15000×g for 20 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

II. Determination

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 400 nm, set zero with distilled water.

2. Standard

Take 100 μL standard solution and add it to 400 μL Solution III to get 1 μmol/mL standard solution. Dilute the solution for 10 times to 100 nmol/mL, and dilute it to 50, 25, 12.5, 6.25 nmol/mL with the distilled water. Detect the standard solutions of 100, 50, 25, 12.5 6.25 and 0 nmol/mL.

3. Add reagents with the following list:

Reagent	Test Tube (T)	Contrast Tube (C)	Standard Tube (S)
Solution I (μL)	400	-	
Solution II (μL)	500	500	
Sample (μL)	100	100	
Mix thoroughly and incubate the reaction for 30 minutes at 37℃ water bath, then take the reaction soulution in a boiling water bath for 5 minutes immediately (tightly close to prevent moisture loss), flowing water to cool.			
Solution I (μL)		400	
Mix thoroughly, centrifuge at 8000 ×g for 5 minutes 4℃ and take the supernatant.			
Supernatant (μL)	500	500	
Standard (μL)			500
Solution III (μL)	1000	1000	1000

Mix thoroughly and stand at room temperature for 2 minutes. Detect the absorbance of each tube at 400 nm and noted as A_T, A_C, A_S and A_B. Calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. Each test tube should be provided with one contrast tube.

III. Calculate:

1. Standard curve

Standard curve established: According to the concentration of the standard tube (y) and absorbance $\Delta A_S = A_S - A_B$ (x), establish standard curve. Add ΔA into the standard curve, and calculate the amount of product generated by the sample (nmol/mL).

2. Calculation

1) Tissue protein concentration

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

β-GC Activity(U/mg prot)=(y×Vrv)÷(Vs×Cpr)÷T=20×y÷Cpr

2) Tissue weight

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation

of 1 nmol of p-nitrophenol in the reaction system per hour every g sample.
β-GC Activity(U/g weight)= (y×Vrv)÷(W×Vs÷Ve)÷T=20×y÷W
3) Bacteria or cultured cells
Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of p-nitrophenol in the reaction system per hour every 10^4 bacteria or cells.
β-GC Activity(U/10^4 cell)=(y×Vrv)÷(1000×Vs÷Ve)÷T=0.02×y

Cpr: Supernatant sample protein concentration (mg/mL);
Vrv: Total reaction volume, 1 mL;
Vs: Supernate volume, 0.1 mL;
Ve: Extract solution volume, 1 mL;
T: Reaction time (min), 30 minutes = 0.5 hour;
W: Sample weight, g;
1000: 10 million cells or bacteria;

Recent Products Citations：

[1] Yu Qian,Jiale Song,Peng Sun,et al. Lactobacillus casei Strain Shirota Enhances the In Vitro Antiproliferative Effect of Geniposide in Human Oral Squamous Carcinoma HSC-3 Cells. Molecules. 2018;(IF3.06)

[2] Zhang Q A, Shi F F, Yao J L, et al. Effects of ultrasound irradiation on the properties of apricot kernels during accelerated debitterizing[J]. RSC Advances, 2020, 10(18): 10624-10633.

References：

[1] Faria M L, Kolling D, Camassola M, et al. Comparison of Pennicillium echinulatum and Trichoderma reesei cellulases in relation to their activity against various cellulosic substrates[J]. Biores. Technol, 2008, 99: 1417-1424.

Related Products：

NA0840/NA0598 Glucogen Content Assay Kit
NA0838/NA0596 β-1,3-glucanase(β-1,3-GA) Activity Assay Kit
NA0691/NA0450 Trehalase Activity Assay Kit