SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

β-glucosidase (β-GC) Assay Kit

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

Operation Equipment: Microplate Reader/ Spectrophotometer

Catalog Number: AK0206

Size:100T/48S

Components:

Extract solution: Liquid 100 mL×1. Storage at 4℃.

Solution I: Powder×1. Storage at -20℃. Add 12 mL of distilled water to per bottle before use and dissolve it fully. The left reagent store at -20℃.

Solution Ⅱ: Liquid 15 mL×1. Storage at 4℃.

Solution Ⅲ: Liquid 15 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.

Microplate reader or spectrophotometer, centrifuge, water-bath, transferpettor, micro glass cuvette/96 well flat-bottom plate, 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. 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%, 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 Extract solution to 0.2 g of tissue, and fully homogenized on ice bath. 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 microplate reader or 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)	120	-	
Solution Ⅱ (μL)	150	150	
Sample (μL)	30	30	
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. Mix thoroughly (keep the concentration unchanged).			
Solution I (μL)		120	
Mix thoroughly, centrifuge at 8000 ×g for 5 minutes 4℃ and take the supernatant. Add the following reagents to EP tube or 96 well flat-bottom plate:			
Supernatant (μL)	70	70	
Standard (μL)			70
Solution Ⅲ (μL)	130	130	130

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, 0.3 mL;

Vs: Supernate volume, 0.03 mL;

Ve: Extract solution volume, 1 mL;

W: Sample weight, g;

1000: 10 million cells or bacteria;

T: Reaction time (min), 30 minutes = 0.5 hour.

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：

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