β-glucosidase (β-GC) Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC2560

Size:50T/24S

Components:

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

Reagent I: Powder×2. Storage at -20°C. Add 10 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 25 mL×1. Storage at 2-8°C.

Reagent III: Liquid 80 mL×1. Storage at 2-8°C.

Standard: Liquid 1 mL×1. Storage at 2-8°C.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 p-nitrophenol 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.

Spectrophotometer, desk centrifuge, water bath/constant temperature incubator, sonicator, transferpettor, 1 mL glass cuvette, mortar/homogenizer, ice 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 200W, working time 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 15000×g for 20 minutes at 4°C to remove insoluble materials and take the supernatant on ice before testing.

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2. Tissue

According to the ratio of tissue mass (g): volume of extraction solution (mL) of 1:5~10 (It is recommended to weigh approximately 0.2g of tissue and add 1mL of extraction solution) and homogenize in an ice bath. Centrifuge at 15000g for 20min at 4°C, remove supernatant and place on ice for measurement.

II. Determination

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 400 nm, set 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(μL)	Test Tube (T)	Contrast Tube (C)	Standard Tube (S)
Reagent I	400	-	
Reagent II	500	500	
Sample	100	100	
Mix thoroughly and incubate the reaction for 30 minutes at 37℃ water bath/constant temperature incubator, 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).			
Reagent I		400	
Mix thoroughly, centrifuge at 8000 ×g for 5 minutes 4℃ and take the supernatant.			
Supernatant	500	500	
Standard			500
Reagent III	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. Standard curve and blank tube only need to be measured once or twice.

III. Calculate:

1. 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. 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 milligram protein.

β-GC Activity(U/mg prot)=$(x \times Vrv) \div (Vs \times Cpr) \div T = 20 \times x \div Cpr$

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 gram sample.

β-GC Activity(U/g weight)= (x×Vrv)÷(W×Vs÷Ve)÷T=20×x÷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)=(x×Vrv)÷(1000×Vs÷Ve)÷T=0.02×x

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.

Note:

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

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:

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