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: Microplate Reader/Spectrophotometer

Catalog Number: BC2565

Size:100T/48S

Components:

Extract solution: Liquid 100 mL×1. Storage at 2-8℃.

Reagent I: Powder×2. Storage 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. Storage at 2-8℃.

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

Standard: Liquid 1 mL×1. Storage at 2-8℃. 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/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. 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 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. Tissues

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 spectrophotometer/microplate reader for more than 30 minutes, adjust the 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(μL)	Test Tube (T)	Contrast Tube (C)	Standard Tube (S)
Reagent I	120	-	
Reagent II	150	150	
Sample	30	30	
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		120	
Mix thoroughly, centrifuge at 8000 ×g for 5 minutes 4°C and take the supernatant. Add the following reagents to EP tube or 96 well flat-bottom plate:			
Supernatant	70	70	
Standard			70
Reagent III	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. 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×Vrv)÷(Vs×Cpr)÷T=20×x÷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, 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.

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

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