β-galactosidase (β-GAL) Assay Kit

Note: The reagents in this product are subject to change, please note and follow these instructions.

Operation Equipment: Spectrophotometer

Catalog Number: BC2580

Size:50T/24S

Components:

Extract solution: Liquid 50 mL×1. Storage at 2-8℃.

Reagent I: Powder×2. Storage at -20℃. Add 2.67 mL distilled water to each bottle before use, fully dissolved. It can be divided into small tubules 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 80 mL×1. Storage at 2-8℃.

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

Product Description

β-galactosidase (β-GAL, EC 3.2.1.23) is an enzyme found broadly in animals, plants, microorganisms and cultured cells, which can catalyze the hydrolysis of β-galactosyl bonds and also has the function of transglycosylation. β-GAL can release stored energy for the rapid growth of plants, also catalyzes the degradation of polysaccharides, glycoproteins, and galactose terminal galactose residues in normal polysaccharide metabolism, cell wall component metabolism and during aging cell wall to release free galactose.

β-GAL can catalyze the p-nitrophenyl-β-pyran galactoside to p-nitrophenol. The product has characteristic of absorption at 400 nm. In this kit, the β-GAL 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 baths/constant temperature incubators, ultrasonic cell disruptor, 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. Suggest add 1 mL of Extract solution to 5 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 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

2. Tissue

The tissue mass (g): volume of extraction solution (mL) should be 1:5~10.(It is recommended

to weigh approximately 0.1g of tissue and add 1mL of extraction solution), homogenise in an ice bath. 15000g centrifuged at 4°C for 10min, supernatant removed and placed on ice for 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 working solution: dilute 5 μmol/mL p-nitrophenol solution to 200, 100, 50, 25, 12.5, 6.25, 0 (Blank tube) nmol/mL with distilled water.
3. Add reagents with the following list:

Reagent(μL)	Test Tube (T)	Contrast Tube (C)	Standard Tube (S)
Reagent I	200	-	
Distilled water		200	
Reagent II	250	250	
Sample	50	50	
Mix thoroughly and incubate the reaction for 30 minutes at 37℃ water bath/constant temperature foster box.			
Standard			500
Reagent III	1000	1000	1000

Mix thoroughly. 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

A standard curve was created from the absorbance (x, ΔAs) and concentration (y, nmol/mL) of the standard tube, and ΔA (x, ΔAt) was brought into the standard curve to calculate the amount of product y (nmol/mL) generated by the sample.

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-GAL 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 gram sample.

β-GAL Activity(U/g)= (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-GAL Activity(U/10^4 cell)=(y×Vrv)÷(500×Vs÷Ve)÷T=0.04×y

Cpr: Supernatant sample protein concentration (mg/mL);
Vrv: Total reaction volume, 0.5 mL;
Vs: Supernate volume, 0.05 mL;
Ve: Extract solution volume, 1 mL;
T: Reaction time (min), 30 minutes = 0.5 hour;
W: Sample weight, g;
500: 5 million cells or bacteria.

Note:

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

Recend Product Citations:

[1] Shuang Li, Junkun Zhan, Yanjiao Wang,et al. Exosomes from hyperglycemia-stimulated vascular endothelial cells contain versican that regulate calcification/senescence in vascular smooth muscle cells. Cell & Bioscience. September 2018;(IF3.405)

[2] Dongjie Jia,Fei Shen,Yi Wang,et al. Apple fruit acidity is genetically diversified by natural variations in three hierarchical epistatic genes: MdSAUR37, MdPP2CH and MdALMTII. Plant Journal. May 2018;(IF5.726)

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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