β-galactosidase (β-GAL) Assay Kit

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

Operation Equipment: Microplate Reader/Spectrophotometer

Catalog Number: NA0443

Size:100T/48S

Components:

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

Solution I: Powder×1. Storage at -20℃. Add 2.55 mL of distilled water to per bottle before use and dissolve it fully. The left reagent store at -20℃.

Solution II: Liquid 4 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-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.

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 5 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 0.1 g of tissue, and fully homogenized on ice bath. Centrifuge at 15000×g for 20 minutes at 4°C to remove insoluble materials, and take the supernatant on ice before testing.

II. Determination

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

2. Standard

Dilute the solution to 200, 100, 50, 25, 12.5, 6.25, 0 nmol/mL with distilled water.

3. Add reagents with the following list:

Reagent	Test Tube (T)	Contrast Tube (C)	Standard Tube (S)
Solution I (μL)	25	-	-
Distilled water (μL)	-	25	-
Solution II (μL)	35	35	-
Sample (μL)	10	10	-
Mix thoroughly and incubate the reaction for 30 minutes at 37°C water bath.			
Standard (μL)	-	-	70
Solution III (μL)	130	130	130

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.

III. Calculate:

1. Standard curve

Standard curve established: According to the concentration of the standard tube (y nmol/mL) 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-GAL Activity(U/mg prot)=(y×Vrv)÷(Vs×Cpr)÷T=14×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-GAL Activity(U/g)= (y×Vrv)÷(W×Vs÷Ve)÷T=14×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=0.028×y

Cpr: Supernatant sample protein concentration (mg/mL);

Vrv: Total reaction volume, 0.07 mL;

Vs: Supernate volume, 0.01 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.

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：

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