β-galactosidase Assay Kit (Visible Spectrophotometry)

Description

β-GAL (EC 3.2.1.23) is widely found in animals, plants, microorganisms, and cultured cells. It catalyzes the hydrolysis of β-galactosidic bonds in β-galactoside compounds and also exhibits transgalactosylation activity.
β-GAL not only releases stored energy to support rapid plant growth, but also participates in normal polysaccharide metabolism, cell wall component metabolism, and cell wall degradation during senescence. In these processes, it catalyzes the hydrolysis of terminal galactose residues in polysaccharides, glycoproteins, and galactolipids, thereby releasing free galactose.

Detection Principle

β-GAL hydrolyzes p-nitrophenyl-β-D-galactopyranoside to produce p-nitrophenol, which has a maximum absorption peak at 400 nm. The β-GAL activity is calculated by measuring the rate of increase in absorbance.

Packing

Taking 50T/24S packing for example:

Components	Packing	Storage
CB0105V-ES	50 mL x 1	4 °C
CB0105V-A	1 vial (powder) x 1	Store at -20 °C. Before use, add 5 mL of distilled water to each vial and dissolve completely. Any unused reagent should be stored at -20 °C.
CB0105V-B	15 mL x 1	4 °C
CB0105V-C	80 mL×1	4 °C
CB0105V-Standard (5μmol/mL)	1 mL×1	4 °C

Instructions

I. Required Equipment & Materials:

Visible spectrophotometer, benchtop centrifuge, water bath, adjustable pipettes, 1 mL glass cuvettes, mortar/homogenizer, ice, and distilled water.

II. Crude Enzyme Extraction:

1. Bacteria or cultured cells:

First collect bacteria or cells into a centrifuge tube and centrifuge to remove the supernatant. According to the number of bacteria or cells (10^4), add CB0105V-ES at a ratio of 500–1000:1 (mL) (recommended: add 1 mL CB0105V-ES to 5 × 10^6 bacteria or cells). Disrupt the bacteria or cells by ultrasonication (ice bath, 20% power or 200 W, sonicate for 3 s with 10 s intervals, repeat 30 times). Centrifuge at 15,000 g for 10 min at 4°C, collect the supernatant, and keep it on ice for analysis.

2. Tissue:

According to tissue weight (g), add CB0105V-ES at a ratio of 1:5–10 (mL) (recommended: weigh about 0.1 g tissue and add 1 mL CB0105V-ES), then homogenize in an ice bath. Centrifuge at 15,000 g for 10 min at 4°C, collect the supernatant, and keep it on ice for analysis.

III. Assay Procedure

1. Preheat the spectrophotometer for at least 30 minutes, set the wavelength to 400 nm, and use distilled water to zero the instrument.
2. Preparation of standards: Dilute the standard solution with distilled water to concentrations of 250, 125, 62.5, 31.25, 15.625, and 0 nmol/mL.
3. Sample measurement (add the following reagents sequentially into EP tubes):

	Sample Tube（μL）	Control Tube（μL）	Standard Tube（μL）
CB0105V-A	200		
Distilled Water		200	
CB0105V-B	250	250	
Sample	50	50	
Mix thoroughly and incubate in a 37 °C water bath for 30 minutes			
Standard solution			500
CB0105V-C	1000	1000	1000
Mix thoroughly, measure the absorbance at 400 nm (A), and calculate ΔA = A_sample − A_control; each sample must have a corresponding control.			

IV. Calculation of β-GAL Activity

1. Establishment of the Standard Curve:

A standard curve is generated using the absorbance of the standard tubes (x, subtracting the OD value of the zero-concentration standard) and concentration (y, nmol/mL).

The ΔA value is then applied to the standard curve to calculate the amount of product generated in the sample, expressed as y (nmol/mL).

2. Calculation of β-GAL Activity:

(1) Based on protein concentration:

Definition: One unit of enzyme activity is defined as the amount of enzyme that produces 1 nmol of p-nitrophenol per hour per mg of tissue protein.

β-GAL activity (U/mg prot) = (y × V1) ÷ (V2 × Cpr) ÷ T = 20 × y ÷ Cpr

Protein concentration (Cpr) must be measured separately.

(2) Based on sample mass:

Definition: One unit of enzyme activity is defined as the amount of enzyme that produces 1 nmol of p-nitrophenol per hour per gram of tissue.

β-GAL activity (U/g tissue) = (y × V1) ÷ (W × V2 ÷ V3) ÷ T = 20 × y ÷ W

(3) Based on bacterial or cell density:

Definition: One unit of enzyme activity is defined as the amount of enzyme that produces 1 nmol of p-nitrophenol per hour per 10^4 cells or bacteria.

β-GAL activity (U/10^4 cells) = (y × V1) ÷ (500 × V2 ÷ V3) ÷ T = 0.04 × y

Note:

Cpr: Protein concentration of the sample (mg/mL), measured separately

V1: Total reaction volume (0.5 mL)

V2: Volume of sample added to the reaction system (0.05 mL)

V3: Volume of extraction solution added (1 mL)

W: Sample weight (g)

500: Total number of cells or bacteria (5 × 10^6)

T: Reaction time (0.5 h)

Precautions

1. The extraction buffer contains components that denature proteins; therefore, when calculating protein concentration, the protein must be re-extracted for measurement.
2. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
3. Please wear a lab coat and disposable gloves.

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