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α-galactosidase (α-GAL) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: AK0205

Size:50T/24S

Components:

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

Solution I: Powder×1. Storage at -20℃ . Add 5 mL of distilled water to per bottle before use and dissolve it fully. The left reagent store at -20℃ .

Solution Ⅱ: Liquid 15 mL×1. Storage at 4℃ .

Solution Ⅲ: Liquid 80 mL×1. Storage at 4℃ .

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

Product Description

α-galactosidase (α-GAL, EC 3.2. 1.22) is an enzyme found broadly in animals, plants, microorganisms and cultured cells. α-GAL catalyze the hydrolysis of α-galactosyl bonds specifically, and mainly participating in the degradation of galactosides such as raffinose, stachyose, melibiose, and galactomannan. α-GAL is crucial for the germination of plant seeds. During the initial stage of seed germination, the D-galactose produced by its catalysis is rapidly transformed and consumed by the glycolytic pathway, which provides the initial source of energy for seed germination. In the later stage, it mainly participates in cell wall storage Polysaccharide hydrolysis.

α-GAL can catalyze the p-nitrophenyl-α-pyran galactoside to p-nitrophenol. The product has characteristic of absorption at 400 nm. In this kit, the α-GAL activity is quantified by measuring the increase in the color development at 400 nm.

Reagents and Equipment Required but Not Provided.

Centrifuge, water-bath, transferpettor, spectrophotometer, 1 mL glass cuvette, 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℃ to remove insoluble materials, and take the supernatant on ice before 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

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)	200	-	
Distilled water (μL)		200	
Solution Ⅱ (μL)	250	250	
Sample (μL)	50	50	
Mix thoroughly and incubate the reaction for 30 minutes at 37℃ water bath.			
Standard (μL)			500
Solution Ⅲ (μL)	1000	1000	1000

Mix thoroughly. Place at room temperature for 2 min. 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.

α-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 g sample.

α-GAL Activity(U/g weight)= (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.

α-GAL Activity(U/10^4 cell)=(y×Vrv)÷(1000×Vs÷Ve)÷T=0.02×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;
1000: 10 million cells or bacteria.

Related Products:

AK0291/AK0290 Glucogen Content Assay Kit
AK0556/AK0555 β- 1,3-glucanase(β- 1,3-GA) Activity Assay Kit
AK0218/AK0217 Trehalase Activity Assay Kit