α-Galactosidase (α-GAL) Activity 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: BC2575

Size:100T/48S

Components:

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

Reagent I: Powder×2. Storage at -20℃. Add 1.25 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 4 mL×1. Storage at 2-8℃.

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

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 p-nitrophenol 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.

Spectrophotometer/microplate reader, desk centrifuge, water bath/ constant temperature incubator, 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 5 million of bacteria or cells. Use ultrasonication to splitting bacteria and cells (place 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. 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 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 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	25	-	-
Distilled water	-	25	-
Reagent II	35	35	-
Sample	10	10	-
Mix thoroughly and incubate the reaction for 30 minutes at 37℃ water bath/constant temperature foster box.			
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

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

α-GAL Activity(U/mg prot)=(x×Vrv)÷(Vs×Cpr)÷T=14×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.

α-GAL Activity(U/g weight)= (x×Vrv)÷(W×Vs÷Ve)÷T=14×x÷W

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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)=(x×Vrv)÷(500×Vs÷Ve)÷T=0.028×x

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.

Note:

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

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