

## $\alpha$ -galactosidase ( $\alpha$ -GAL) Activity Assay Kit

**Note:** The reagents of this product are subject to change. Please note and strictly follow this instruction.

**Operation Equipment:** Spectrophotometer

**Catalog Number:** BC2570

**Size:** 50T/24S

### Components:

**Extract solution:** Liquid 50 mL×1. Storage at 2-8°C.

**Reagent I:** Powder×2. Storage at -20°C. Add 2.67 mL distilled water to each bottle before use, fully dissolved. It can be divided into small tubules and stored at -20°C for 4 weeks. Avoid repeating freeze thaw cycles.

**Reagent II:** Liquid 15 mL×1. Storage at 2-8°C.

**Reagent III:** Liquid 80 mL×1. Storage at 2-8°C.

**Standard:** Liquid 1 mL×1. Storage at 2-8°C. 5  $\mu$ mol/mL p-nitrophenol solution.

### Product Description

$\alpha$ -Galactosidase ( $\alpha$ -GAL, EC 3.2.1.22) is an enzyme found broadly in animals, plants, microorganisms and cultured cells.  $\alpha$ -GAL catalyze the hydrolysis of  $\alpha$ -galactosyl bonds specifically, and mainly participating in the degradation of galactosides such as raffinose, stachyose, melibiose, and galactomannan.  $\alpha$ -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.

$\alpha$ -GAL can catalyze the p-nitrophenyl- $\alpha$ -pyran galactoside to p-nitrophenol. The p-nitrophenol has characteristic of absorption at 400 nm. In this kit, the  $\alpha$ -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 bath/ constant temperature incubator, cell sonicator, 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 20 minutes at 4°C 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 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°C water bath/constant temperature foster box.			
Standard			500
Reagent III	1000	1000	1000

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,  $\Delta A_S$ ) and concentration (x, nmol/mL) of the standard tube, and  $\Delta A$  (y,  $\Delta A_T$ ) 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.

$$\alpha\text{-GAL Activity (U/mg prot)} = (x \times V_{rv}) \div (V_s \times C_{pr}) \div T = 20 \times x \div C_{pr}$$

#### 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.

$$\alpha\text{-GAL Activity (U/g weight)} = (x \times V_{rv}) \div (W \times V_s \div V_e) \div T = 20 \times x \div W$$

#### 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.

$$\alpha\text{-GAL Activity(U/10}^4\text{ cell)}=(x\times V_{rv})\div(1000\times V_s\div V_e)\div T=0.02\times x$$

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.

**Note:**

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

**Related Products:**

BC0340/BC0345	Glucogen Content Assay Kit
BC0360/BC0365	$\beta$ -1,3-glucanase( $\beta$ -1,3-GA) Activity Assay Kit
BC2510/BC2515	Trehalase Activity Assay Kit