

# $\alpha$ -Mannosidase ( $\alpha$ -man) Activity Assay Kit

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

**Operation Equipment:** Ultraviolet spectrophotometer/Microplate reader

**Catalog Number:** BC5045

**Size:** 100T/96S

## Components:

Reagent	Size	Storage
Extract solution	Liquid 110mL×1	4°C
Reagent I	Liquid 15 mL×1	4°C
Reagent II	powder×2	-20°C
Reagent III	Liquid 8 mL×1	4°C
Reagent IV	Liquid 1.5 mL×1	4°C
标准品	Liquid 1mL×1 支	4°C保存

Solution preparation:

1. Reagent II: Before use, add 0.5mL reagent IV to each to dissolve it, and store the dissolved reagent in aliquots at -20°C, which can be stored for 4 weeks.
2. Standard: 5 mmol/L standard solution.

## Product Description:

$\alpha$ -Mannosidase is widely distributed and has many kinds. It is found in eukaryotic cytoplasm, endoplasmic reticulum, Golgi apparatus, and lysosome. Different types and functions of  $\alpha$ -Man participate in the modification process of N-glycans.

$\alpha$ -Mannosidase reacts with a specific substrate, and the product has a characteristic absorption peak at 405nm. The  $\alpha$ -man activity can be calculated according to the rate of change in absorbance.

## Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, desk centrifuge, pipette, constant temperature incubator/water bath, micro quartz cuvette/96 well UV well plate, mortar/homogenizer, ice and distilled water.

## Procedure

### I. Sample preparation:

1. Tissue sample:

according to the proportion of tissue weight (g): extraction solution volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of extraction solution and fully homogenized on ice bath. Centrifuge at 12000 ×g for 10 minutes at 4°C to remove insoluble materials, and take the supernatant on ice before testing.

2. Bacteria or cells:

Collecting bacteria or cells into the centrifuge tube, suggested 5 million with 1 mL of extraction

solution. Use ultrasonication to splitting bacteria and cells (placed on ice, ultrasonic power 200w, working time 3 seconds, interval 7 seconds, repeat for 30 times). Centrifuge at 12000 ×g for 10 minutes at 4°C to remove insoluble materials, and take the supernatant on ice for testing.

3. Serum: Detect directly.

## II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 405 nm, set zero with distilled water.
2. Dilute 5mmol/L maltose standard solution with distilled water to 0.625, 0.3125, 0.15625, 0.078, 0.039, 0.0195, 0.01mmol/L standard solutions.
3. Add reagents with the following list:

(1) Enzymatic reaction (In 1.5 mL EP tube)

Reagent (μL)	Contrast tube(c)	Test tube(t)	Standard tube(s)	Blank tube(b)
Sample	25	25	-	-
Reagent I	110	125	125	125
Reagent II	15	-	-	-
Standard	-	-	25	-
Distilled water	-	-	-	25
Mix thoroughly. 37°C water bath for 10 minutes.				
Reagent III	50	50	50	50
Mix thoroughly. Pipette 200 μL into a micro cuvette or 96-well plate, measure the absorbance at 405 nm, and record them as Ac, At, As, and Ab. Calculate $\Delta A = A_t - A_c$ , $\Delta A_s = A_s - A_b$ .				

Note: Blank tube only need to test 1~2 times. and the standard curve only needs to be tested 1-2 times.

## III. Calculations:

1. Standard curve

Take the concentration of each standard solution as x-axis, and the corresponding  $\Delta A_s$  is y-axis. Then the linear regression equation  $y = kx + b$  is obtained. Bring  $\Delta A$  into the equation to get x (μmol/mL).

2. α-man activity

A. Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 mmol p-nitrophenol per minute every milligram protein.

$$\alpha\text{-man (U/mg prot)} = x \times V_S \div (V_S \times C_{pr}) \div T \times F = x \times 0.1 \div C_{pr} \times F$$

B. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 mmol p-nitrophenol per minute every gram tissue.

$$\alpha\text{-man (U/g weight)} = x \times V_S \div (W \times V_S \div V_E) \div T \times F = x \times 0.1 \div W \times F$$

C. Cell amount

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 mmol p-nitrophenol per minute every  $10^4$  bacteria or cells.

$$\alpha\text{-man (U/}10^4\text{ cell)} = \frac{x \times V_S}{(\text{cells } (10^4) \times V_S \div V_E) \div T \times F} \times 0.1 \div \text{cells } (10^4) \times F$$

#### D. Serum

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 mmol p-nitrophenol per minute per milliliters.

$$\alpha\text{-man (U/ mL)} = \frac{x \times V_S \div V_S \div T \times F}{x \times 0.1 \times F}$$

$V_S$ : Add sample volume, 0.025 mL;

$V_E$ : Extract solution volume, 1 mL;

T: Reaction time, 10 min;

Cpr: Protein concentration of sample, mg/mL;

W: Sample weight, g;

F: Dilution ratio.

#### Note:

1. If the measured absorbance value  $A > 1.5$  or  $\Delta A > 0.5$ , it is recommended to dilute the sample before measuring, and multiply the dilution factor in the calculation formula; if the measured absorbance value is low or close to the blank OD value, it is recommended to increase the sample volume before performing the measurement.

#### Experimental example

1. Take 0.1 g of rabbit liver tissue, add 1 mL extract solution, and homogenize in ice bath. Centrifuge at 12000 g, 4°C for 10 min. Take the supernatant for test. Following the measurement procedure. Calculate  $\Delta A = A_2 - A_1 = 0.404 - 0.309 = 0.095$ .  $y = 1.3642x + 0.0037$ ,  $x = 0.0669$ . Calculate the activity according to the formula:

$$\alpha\text{-man activity (mmol/min/g weight)} = \frac{x \times 0.1 \div W \times F}{0.0069} = 0.0069 \text{ U/g weight.}$$

#### Related products

BC0360/BC0365  $\beta$ -1,3-glucanase( $\beta$ -1,3-GA) Activity Assay Kit

BC2550/BC2555  $\alpha$ -glucosidase( $\alpha$ -GC) Activity Assay Kit

BC2560/BC2565  $\beta$ -glucosidase( $\beta$ -GC) Activity Assay Kit

BC2570/BC2575  $\alpha$ -galactosidase( $\alpha$ -GAL) Activity Assay Kit

BC2580/BC2585  $\beta$ -galactosidase( $\beta$ -GAL) Activity Assay Kit

