# α-Mannosidase (α-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℃ |
| Reagent I | Liquid 15 mL×1 | 4℃ |
| Reagent II | powder×2 | -20℃ |
| Reagent III | Liquid 8 mL×1 | 4℃ |
| Reagent IV | Liquid 1.5 mL×1 | 4℃ |
| Standard | Liquid 1mL×1 支 | 4℃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：**

α-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 α-Man participate in the modification process of N-glycans.

α-Mannosidase reacts with a specific substrate, and the product has a characteristic absorption peak at 405nm. The α-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℃ 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℃ 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℃ 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 ΔA=At-Ac, ΔAs=As-Ab. | | | | |

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 ΔA 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.

α-man (U/mg prot)＝$x \times V_S \div (V_S \times Cpr) \div T \times F = x \times 0.1 \div Cpr \times F$

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.

α-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.

α-man (U/$10^4$ cell)＝x×$V_S$÷（cells（$10^4$）×$V_S$÷$V_E$）÷T×F x×0.1÷cells（$10^4$）×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 minute per milliliters.

α-man (U/ mL)＝x×$V_S$÷$V_S$÷T×F= x×0.1×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 ΔA>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℃ for 10 min. Take the supernatant for test. Following the measurement procedure. Calculate ΔA=A2-A1=0.404-0.309=0.095. y=1.3642x+ 0.0037, x=0.0669. Calculate the activity according to the formula: α-man activity（mmol/min/g weight）= x×0.1÷W×F =0.0069 U/g weight.

**Related products**

BC0360/BC0365β-1,3-glucanase(β-1,3-GA) Activity Assay Kit

BC2550/BC2555α-glucosidase(α-GC) Activity Assay Kit

BC2560/BC2565β-glucosidase(β-GC) Activity Assay Kit

BC2570/BC2575α-galactosidase(α-GAL) Activity Assay Kit

BC2580/BC2585β-galactosidase(β-GAL) Activity Assay Kit