α-Mannosidase (α-man) Activity Assay Kit

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

Operation Equipment: Ultraviolet spectrophotometer

Catalog Number: BC5054

Size: 50T/24S

Components:

Reagent	Size	Storage
Extract solution	Liquid 60mL×1	4℃
Reagent I	Liquid 50 mL×1	4℃
Reagent II	powder×2	-20℃
Reagent III	Liquid 20 mL×1	4℃
Reagent IV	Liquid 3 mL×1	4℃
Standard	Liquid 1 mL×1	4℃

Solution preparation:

1. Reagent II: Before use, add 1mL reagent IV to each to dissolve it, and store the dissolved reagent in aliquots at -20°C, which can be stored for 2 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, desk centrifuge, constant temperature incubator/water bath, pipette, 1 mL quartz cuvette, 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 for 30 minutes, adjust wavelength to 405 nm, set zero with distilled water.
2. Dilute 5 mmol/L maltose standard solution with distilled water to 0.625、0.3125、0.15625、0.078、0.039, 0.0195, 0.01, 0.005 mmol/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	125	125	-	-
Reagent I	550	625	625	625
Reagent II	75	-	-	-
Standard	-	-	125	-
Distilled water	-	-	-	125
Mix thoroughly. 37℃ water bath for 10 minutes.				
Reagent III	250	250	250	250
Mix thoroughly. 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 ΔA_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, 0.125 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>0.1, 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, 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. Standard Curve: y=2.0294x+ 0.0092, x= 0.0422. Calculate the activity according to the formula:

α-man activity（mmol/min/g weight）= x×0.1÷W×F =0.0422 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