N-Acetyl-β-D-Glucosidase (NAG) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC4295

Size: 100T/48S

Components:

Extract solution: Liquid 60 mL×1, store at 4℃;

Reagent I: Liquid 10 mL×1, store at 4℃;

Reagent II: Powder×2, store at -20℃. Add 1 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 III: Liquid 30 mL×1, store at 4℃;

Standard: Liquid 1 mL×1, 5 μmol/mL p-nitrophenol solution, store at 4℃.

Product Description:

N-acetyl-β-D-glucosidase (NAG, EC 3.2.1.52) is widely distributed in various tissues. It is an intracellular lysosomal enzyme. The activity of Nag can be used for the early diagnosis of tubulointerstitial nephritis, urinary tract infection, diabetic nephropathy syndrome, hypertensive nephropathy, rejection after kidney transplantation and nephrotic syndrome.

NAG decomposes N-β-acetylglucosamine to produce p-nitrophenol. It has a maximum absorption peak at 400 nm. NAG activity was calculated by measuring the change of absorbance at 400 nm.

Required but Not Provided:

Spectrophotometer/microplate reader, balance, desk centrifuge, water bath/constant temperature foster box, ultrasonic cell disruptor, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ice and distilled water.

Procedure

I. Preparation of standard samples:

1. Tissue: according to the ratio of mass (g): extraction volume (mL): 1:5-10 to add the extract solution. It is suggested that add 1 mL of extract solution to 0.1 g of tissue. Homogenize on ice. Centrifuge at 15000 g 4℃ for 10 min. Take the supernatant on ice for test.
2. Bacteria or cell: according to the ratio of 10^4 cells: extract solution volume (mL) 500-1000:1. It is suggested to take about 500 million bacteria/cell and add 1 mL extraction solution. Bacteria/cell is split by ultrasonication (power 200w, ultrasonic 3s, interval 7s, total time 3 min). Centrifuge at 15000 g 4℃ for 10 min. Take the supernatant on ice for test.
3. Serum (plasma) and other liquid: detect directly.

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 the standard 8 times with distilled water to obtain 0.625 μmol/mL standard solution.

3. Operation table: (add the following reagents in 1.5 mL centrifuge tube in turn)

Reagent (μL)	Test tube (A_T)	Control tube (A_C)	Standard tube (A_S)	Blank tube (A_B)
Reagent I	60	60	60	60
Reagent II	30	-	-	-
Preheat 5min at 37℃				
Distilled water	-	30	30	40
Standard	-	-	10	-
Sample	10	10	-	-
React at 37℃ for 30 min.				
Reagent III	200	200	200	200

Mix thoroughly. Place at room temperature for 2min. Take 200 μL into micro glass cuvette/96 well flat-bottom plate. Measure the absorbance value at 400 nm wavelength. Record as A_T, A_C, A_S, A_B. $\Delta A_S=A_S-A_B$. $\Delta A_T=A_T-A_C$. 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. Protein concentration:

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the generation of 1 nmol p-nitrophenol per minute every mg tissue protein in the reaction system.

NAG (U/mg prot) $=\Delta A_T\div(\Delta A_S\div C_S)\times1000\times V_{SA}\div(Cpr\times V_{SA})\div T=20.83\times\Delta A_T\div\Delta A_S\div Cpr$

2. Sample weight:

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the generation of 1 nmol p-nitrophenol per minute every gram tissue weight in the reaction system.

NAG (U/g weight) $=\Delta A_T\div(\Delta A_S\div C_S)\times1000\times V_{SA}\div(V_{SA}\div V_E\times W)\div T=20.83\times\Delta A_T\div\Delta A_S\div W$

3. Cells

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the generation of 1 nmol p-nitrophenol per minute every 10^4 cells in the reaction system.

NAG (U/10^4 cell) $=\Delta A_T\div(\Delta A_S\div C_S)\times1000\times V_{SA}\div(V_{SA}\times Cells\div V_E)\div T=20.83\times\Delta A_T\div\Delta A_S\div Cells$

4. Liquid volume

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the generation of 1 nmol p-nitrophenol per minute every mL serum in the reaction system.

NAG (U/mL) $=\Delta A_T\div(\Delta A_S\div C_S)\times1000\times V_{SA}\div V_{SA}\div T=20.83\times\Delta A_T\div\Delta A_S$

C_S: Concentration of standard solution: 0.625 μmol/mL;

V_E: Extract solution volume of cells, 1 mL;

V_{SA}: Sample volume, 0.01 mL;
Cpr: Protein concentration, mg/mL;
T: Reaction time, 30 min;
Cells: The number of cells, 10^4 cells as a unit;
W: Sample weight, g;
1000: The conversion coefficient is 1 μmol=1000 nmol.

Note:

1. If the absorbance is greater than 1.5, it is recommended to dilute the sample with the extract for determination.

Experimental examples:

1. Take 0.1 g of rat spleen and add 1 mL of Extract solution for sample processing. The supernatant was diluted 5 times, and then proceeded according to the measurement procedure. After determination with 96 well flat-bottom plate, calculate $\Delta A_T=A_T-A_C=0.582-0.067=0.515$, $\Delta A_S=A_S-A_B=0.268-0.047=0.221$. The enzyme activity is calculated according to the sample mass.
NAG (U/g weight) $=20.83\times\Delta A_T\div\Delta A_S\div W\times5$(dilution times)=2427.02 U/g weight.
2. Take 0.1 g of magnolia leaves and add 1 mL of Extract solution for sample processing. The supernatant was diluted 5 times, and then proceeded according to the measurement procedure. After determination with 96 well flat-bottom plate, calculate $\Delta A_T=A_T-A_C=0.405-0.112=0.293$, $\Delta A_S=A_S-A_B=0.268-0.047=0.221$. The enzyme activity is calculated according to the sample mass.
NAG (U/g weight) $=20.83\times\Delta A_T\div\Delta A_S\div W$=276.162 U/g weight.
3. Take the rabbit serum directly according to the determination procedure. After determination with 96 well flat-bottom plate, calculate $\Delta A_T=A_T-A_C=0.368-0.246=0.122$, $\Delta A_S=A_S-A_B=0.268-0.047=0.221$. The enzyme activity is calculated according to the liquid volume.
NAG (U/mL) $=20.83\times\Delta A_T\div\Delta A_S$=11.4989 U/mL.

Related products:

BC3330/BC3335 Glycogen synthase(GCS) Activity Assay Kit
BC3360/BC3365 UDP-glucose pyrophosphorylase(UGP) Activity Assay Kit
BC4440/BC4445 Hemicellulose Content Assay Kit