SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

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

Cat No: AK0088

Size: 50T/24S

Components:

Extract solution: Liquid 30 mL×1, store at 4℃;

Reagent I: Liquid 20 mL×1, store at 4℃;

Reagent II: Powder×1, store at -20℃ . Add 5 mL distilled water when the solution will be used. The rest of reagent can store at -20℃ for two weeks; Avoid repeated freezing and thawing;

Reagent III: Liquid 60 mL×1, store at 4℃;

Standard: Liquid 1 mL×1, 5 μmol/mL p-nitrophenol solution, store at 4℃ . Before use, dilute the standard 8 times with distilled water to obtain 0.625 μmol/mL standard solution.

Product Description:

N-acetyl-β-D-glucosidase (NAG) 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, balance, centrifuge, water-bath, transferpettor, 1 mL glass cuvette, EP tube, mortar/ homogenizer and distilled water.

Protocol

I. Preparation:

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 300w, 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 procedure:

1. Preheat ultraviolet spectrophotometer for 30 min, adjust wavelength to 400 nm, set the counter to zero with distilled water.

2. 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	300	300	300	300
Reagent II	150	-	-	-
Distilled water	-	150	150	200
Standard	-	-	50	-
Sample	50	50	-	-
React at 37℃ for 30 min.				
Reagent III	1000	1000	1000	1000

Mix thoroughly. Place at room temperature for 2min. Measure the absorbance value at 400 nm wavelength. Record as A_T, A_C, A_S, A_B. $\Delta A_S=A_S-A_B$. $\Delta A_T=A_T-A_C$.

III. NAG Calculation:

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, 0.05 mL;

Cpr: Protein concentration, mg/mL;

T: Reaction time, 30 min;

Cells: The number of cells, 10^4 cell 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, it is recommended to dilute the sample with the extract solution 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. Calculate $\Delta A_T=A_T-A_C=0.798-0.042=0.756$, $\Delta A_S=A_S-A_B=0.364-0.006=0.358$. 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)=2199.368 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. Calculate $\Delta A_T=A_T-A_C=0.543-0.120=0.423$, $\Delta A_S=A_S-A_B=0.364-0.006=0.358$. The enzyme activity is calculated according to the sample mass.

NAG (U/g weight) $=20.83\times\Delta A_T\div\Delta A_S\div W=246.120$ U/g weight.

3. Take the rabbit serum directly according to the determination procedure. Calculate $\Delta A_T=A_T-A_C=0.542-0.348=0.194$, $\Delta A_S=A_S-A_B=0.364-0.006=0.358$. The enzyme activity is calculated according to the liquid volume.

NAG (U/mL) $=20.83\times\Delta A_T\div\Delta A_S=11.2878$ U/mL.

Related products：

AK0139/AK0138 Glycogen synthase(GCS) Activity Assay Kit
AK0137/AK0136 UDP-glucose pyrophosphosphprylase(UGP) Activity Assay Kit
AK0061/AK0060 Hemicellulose Content Assay Kit