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: AK0087

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×1, store at -20℃ . Add 2 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 20 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/microplate reader, balance, desk centrifuge, water-bath, transferpettor, micro glass cuvette/96 well flat-bottom plate, 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/microplate reader 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	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 . $\Delta A_S = A_S - A_B$. $\Delta A_T = A_T - A_C$.

III. NAG Calculation:

IV. 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) $= \Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{SA} \div (Cpr \times V_{SA}) \div T = 20.83 \times \Delta A_T \div \Delta A_S \div Cpr$

V. 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) $= \Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{SA} \div (V_{SA} \div V_E \times W) \div T = 20.83 \times \Delta A_T \div \Delta A_S \div W$

VI. 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) $= \Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{SA} \div (V_{SA} \times Cells \div V_E) \div T = 20.83 \times \Delta A_T \div \Delta A_S \div Cells$

VII. 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) $= \Delta A_T \div (\Delta A_S \div C_S) \times 1000 \times V_{SA} \div V_{SA} \div T = 20.83 \times \Delta A_T \div \Delta A_S$

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;

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W: Sample weight, g;

1000: The conversion coefficient is 1 μmol=1000 nmol.

Note:

1. If the absorbance is greater than 2, 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：

AK0139/AK0138   Glycogen synthase (GCS) Activity Assay Kit

AK0137/AK0136   UDP-glucose pyrophosphphprylase(UGP) Activity Assay Kit

AK0061/AK0060   Hemicellulose Content Assay Kit