N-acetyl- β- D-glucosidase(NAG) Activity Assay Kit

Product Code: 111935

N-acetyl-β-D-glucosidase (NAG) Activity Assay Kit - Micro Method

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Product Introduction

NAG is an acidic hydrolase found in lysosomes and is widely present in tissues, body fluids, and cells. It is most abundant in the prostate and renal proximal tubular cells. Changes in NAG activity are closely related to certain pathological states.

NAG degrades β-N-acetylaminoglucoside to produce p-nitrophenol, which has a maximum absorption peak at 400 nm. NAG activity is calculated by measuring the increase in absorbance.

Package List

Size	Code	Component	Quantity
100T	111935.1	Reagent I	1 bottle
100T	111935.2	Reagent II	1 bottle
100T	111935.3	Reagent III	1 bottle
100T	111935.4	Extraction Solution	1 bottle
100T	111935.m	Manual	1 copy

Quality Standards and Safety Information

Raw Material or Package Name	Quality Standard	Main Toxicity
Reagent I	—	—
Reagent II	—	—
Reagent III	—	—
Extraction Solution	—	—

Transportation and Storage

Transportation	Transport with ice packs.
Storage	Store Reagent I at -20°C. Store all other components at 2–8°C.
Shelf Life	180 days.

Instructions

1. Preparation of Crude Enzyme Extract

1.1 Bacteria or Cultured Cells

1. Collect bacteria or cells into a centrifuge tube, centrifuge, and discard the supernatant.
2. Add Extraction Solution according to the ratio of bacteria or cells number (10^4cells) to Extraction Solution volume (mL) = 500–1000:1. Recommended: add 500 × 10^4bacteria or cells to 1 mL Extraction Solution.
3. Disrupt the bacteria or cells by ultrasonication in an ice bath at 20% power or 200 W: sonicate for 3 s, pause for 10 s, and repeat 30 times.
4. Centrifuge at 15000g and 4°C for 10 min.
5. Collect the supernatant and keep it on ice for testing.

1.2 Tissue

1. Add Extraction Solution according to the ratio of tissue mass (g) to Extraction Solution volume (mL) = 1:5–10. Recommended: weigh approximately 0.1 g tissue and add 1 mL Extraction Solution.
2. Homogenize in an ice bath.
3. Centrifuge at 15000g and 4°C for 10 min.
4. Collect the supernatant and keep it on ice for testing.

2. Reagent Preparation

Before use, add 2.5 mL distilled water to each bottle of Reagent I and dissolve thoroughly. Store unused Reagent I at -20°C.

3. Assay Procedure

1. Preheat the spectrophotometer for at least 30 min.
2. Set the wavelength to 400 nm and zero with distilled water.
3. Prepare the assay and control tubes as shown below.

Component	Assay Tube	Control Tube
Reagent I (μL)	25	
Distilled water (μL)	25	
Reagent II (μL)	35	35
Sample (μL)	10	10

Mix quickly and incubate in a 37°C precision water bath for 30 min.

Component	Assay Tube	Control Tube
Reagent III (μL)	130	130

Mix thoroughly and measure the absorbance at 400 nm. Calculate $\Delta A = A_{measured} - A_{control}$. Each assay tube must have one corresponding control tube.

NAG Activity Calculation

The regression equation measured under standard conditions is y = 0.00543x + 0.0083, where x is the standard concentration (nmol/mL) and y is the absorbance value.

1. Serum or Plasma NAG Activity

Unit definition: the production of 1 nmol p-nitrophenol per minute in each mL of serum or plasma is defined as one unit of enzyme activity.

NAG activity (nmol/min/mL) = $[(\Delta A - 0.0083) \div 0.00543 \times V_{total\ reaction}] \div V_{sample} \div T = 61.39 \times (\Delta A - 0.0083)$

2. Calculation Based on Sample Protein Concentration

Unit definition: the production of 1 nmol p-nitrophenol per minute in each mg of tissue protein is defined as one enzyme activity unit.

NAG activity (nmol/min/mg protein) = $[(\Delta A - 0.0083) \div 0.00543 \times V_{total\ reaction}] \div (V_{sample} \times Cpr) \div T = 61.39 \times (\Delta A - 0.0083) \div Cpr$

3. Calculation Based on Sample Fresh Weight

Unit definition: the production of 1 nmol p-nitrophenol per minute in each g of tissue is defined as one enzyme activity unit.

NAG activity (nmol/min/g fresh weight) = $[(\Delta A - 0.0083) \div 0.00543 \times V_{total\ reaction}] \div (W \times V_{sample} \div V_{total\ sample}) \div T = 61.39 \times (\Delta A -$

0.0083) ÷ W

4. Calculation Based on Bacterial or Cell Density

Unit definition: the production of 1 nmol p-nitrophenol per minute by each 10,000 bacteria or cells is defined as one enzyme activity unit.

NAG activity (nmol/min/10^4cells) = [(ΔA - 0.0083) ÷ 0.00543 × $V_{total\ reaction}$] ÷ (500 × V_{sample}÷ $V_{total\ sample}$) ÷ T = 0.123 × (ΔA - 0.0083)

Formula Parameters

$V_{total\ reaction}$	Total volume of the reaction system, 0.5 mL
V_{sample}	Sample volume added to the reaction system, 0.05 mL
$V_{total\ sample}$	Volume of Extraction Solution added, 1 mL
Cpr	Sample protein concentration, mg/mL
W	Sample mass, g
500	Total number of cells or bacteria, 500 × 10,000
T	Reaction time, 30 min

Precautions

> The 100T kit can test 48 samples.