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

Product Code: 111936

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

Product Information

Product Code	111936
Assay Format	Microplate method
Package Size	100T

Product Introduction

N-acetylglucosaminidase, also known as N-acetyl-β-D-glucosaminidase or NAG, is an intracellular lysosomal enzyme found in organs such as the kidney, liver, spleen, and brain. It is present at the highest level in renal proximal tubules.

NAG has a relatively large molecular weight and cannot be filtered by the glomerulus. When kidney damage occurs, NAG is released from cells into the renal tubules, causing urinary NAG to increase significantly. Urinary NAG activity is a highly sensitive and specific indicator of renal tubular lesions and can serve as an early indicator of renal tubular injury, with earlier predictive value than urinary albumin.

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

Package Contents

Code	Component	Quantity
111936.1	Reagent One	1 bottle
111936.2	Reagent Two	1 bottle
111936.3	Reagent Three	1 bottle
111936.4	Extraction Solution	1 bottle
111936.5	Standard	1 vial
111936.m	Manual	1 copy

Quality and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent One	--	--
Reagent Two	--	--
Reagent Three	--	--
Extraction Solution	--	--
Standard	--	--

Transportation and Storage

Item	Condition
Transportation	Transport with ice packs.
Storage	Store Reagent One at -20°C. Store the remaining reagents at 2-8°C.
Shelf Life	180 days

Instructions for Use

1. Solution Preparation

1. For Reagent One, add 2.5 mL distilled water before use and dissolve thoroughly. Store the remaining prepared reagent at -20°C.
2. Before use, dilute the standard 8-fold to obtain a 0.625 umol/L standard solution.

2. Crude Enzyme Extract Preparation

Bacteria or Cultured Cells

1. Collect the bacteria or cells into a centrifuge tube.
2. Centrifuge and discard the supernatant.
3. Add Extraction Solution according to the ratio of bacteria or cell number to Extraction Solution volume: 500-1000 × 10^4cells per 1 mL. It is recommended to add 1 mL Extraction Solution to 500 × 10^4bacteria or cells.
4. Sonicate in an ice bath at 20% power or 200 W. Sonicate for 3 s, pause for 10 s, and repeat 30 times.
5. Centrifuge at 15000 g and 4°C for 10 min.
6. Collect the supernatant and place it on ice for testing.

Tissue

1. Add Extraction Solution according to the ratio of tissue mass to Extraction Solution volume: 1 g tissue to 5-10 mL Extraction Solution. It is recommended to weigh approximately 0.1 g tissue and add 1 mL Extraction Solution.
2. Homogenize in an ice bath.
3. Centrifuge at 15000 g and 4°C for 10 min.
4. Collect the supernatant and place it on ice for testing.

Serum or Plasma

Serum and plasma samples can be tested directly.

3. Assay Procedure

1. Preheat the microplate reader for at least 30 min.
2. Set the wavelength to 400 nm and zero with distilled water.
3. Add the reagents sequentially into tubes or a 96-well plate according to the table below.

Component	Assay Tube	Control Tube	Standard Tube	Blank Tube
Reagent I	25 μL			
Distilled Water	25 μL	25 μL	35 μL	
Reagent II	35 μL	35 μL	35 μL	35 μL
Sample	10 μL	10 μL		
Standard Solution	10 μL			

Mix quickly and incubate at 37°C for 30 min.

Component	Assay Tube	Control Tube	Standard Tube	Blank Tube
Reagent III	130 μL	130 μL	130 μL	130 μL

Mix thoroughly and measure absorbance at 400 nm. Record the values as $A_{assay\ tube}$, $A_{control\ tube}$, $A_{standard\ tube}$, and $A_{blank\ tube}$.

Calculate $\Delta A_{assay} = A_{assay\ tube} - A_{control\ tube}$. Calculate $\Delta A_{standard} = A_{standard\ tube} - A_{blank\ tube}$.

Each assay tube requires one control tube. The blank tube and standard tube only need to be run 1-2 times.

NAG Activity Calculation

1. Calculation by Protein Concentration

Unit definition: one enzyme activity unit is defined as the amount of enzyme that generates 1 nmol of p-nitrophenol per minute in the reaction system per mg of protein.

$$NAG\ (U/mg\ prot) = \Delta A_{assay} \div (\Delta A_{standard} \div C_{standard}) \times 1000 \times V_{sample} \div (Cpr \times V_{sample}) \div T = 20.83 \times \Delta A_{assay} \div \Delta A_{standard} \div Cpr$$

2. Calculation by Sample Mass

Unit definition: one enzyme activity unit is defined as the amount of enzyme that generates 1 nmol of p-nitrophenol per minute in the reaction system per g of sample.

$$NAG\ (U/g\ mass) = \Delta A_{assay} \div (\Delta A_{standard} \div C_{standard}) \times 1000 \times V_{sample} \div (V_{sample} \div V_{total\ sample} \times W) \div T = 20.83 \times \Delta A_{measured} \div \Delta A_{standard} \div W$$

3. Calculation by Cell Number

Unit definition: one enzyme activity unit is defined as the amount of enzyme that produces 1 nmol of p-nitrophenol per minute in the reaction system per 10^4cells.

$$NAG\ (U/10^4 cells) = \Delta A_{measured} \div (\Delta A_{standard} \div C_{standard}) \times 1000 \times V_{sample} \div (cell\ number \times V_{sample} \div V_{total\ sample}) \div T = 20.83 \times \Delta A_{measured} \div \Delta A_{standard} \div cell\ number$$

4. Calculation by Liquid Volume

Unit definition: one enzyme activity unit is defined as the amount of enzyme that catalyzes the formation of 1 nmol of p-nitrophenol per minute in the reaction system per mL of liquid.

$$NAG\ (U/mL) = \Delta A_{measured} \div (\Delta A_{standard} \div C_{standard}) \times 1000 \times V_{sample} \div V_{sample} \div T = 20.83 \times \Delta A_{measured} \div \Delta A_{standard}$$

Formula Parameters

Symbol	Description
$C_{standard}$	Concentration of the standard solution: 0.625 μmol/mL
$V_{total\ sample}$	Extraction Solution volume: 1 mL
V_{sample}	Sample volume added: 0.01 mL
Cpr	Protein concentration of the supernatant: mg/mL
T	Reaction time: 30 min
Cell number	Counted in ten-thousands
W	Sample mass: g
1000	Conversion factor: 1 μmol = 1000 nmol

Precautions

1. Before the formal assay, select 2-3 samples with large expected differences for a preliminary test.
2. The following instruments and supplies must be provided by the user: microplate reader, benchtop centrifuge, water bath, adjustable pipettes, 96-well plate, mortar, ice, and distilled water.
3. This 100T kit can test 48 samples.