Beta-N-Acetyl-Glucosaminidase (NAG) Assay Kit

Catalog No.: abx294051

Size: 50 tests

Detection Range: 0.1 U/L – 80 U/L

Sensitivity: 0.1 U/L

Storage: Store all components at 4°C. Store the Standard in the dark.

Application: For detection and quantification of Beta-N-Acetyl-Glucosaminidase (NAG) activity in serum, plasma, urine, and tissue homogenates.

Introduction

Abbexa's Beta-N-Acetyl-Glucosaminidase (NAG) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Beta-N-Acetyl-Glucosaminidase (NAG) activity. Beta-N-Acetyl-Glucosaminidase (NAG) catalyzes the hydrolysis of the provided substrate, releasing p-nitrophenol. Adding alkaline solution produces a reaction product with an absorbance maximum at 400 nm. The intensity of the color is proportional to the Beta-N-Acetyl-Glucosaminidase (NAG) activity, which can then be calculated.

Kit components

1. Reagent A: 40 ml
2. Reagent B: 6 ml
3. Substrate: 1 vial
4. Standard (3 mmol/L): 2 ml
5. Stop Solution: 2 × 60 ml

Materials required but not provided

1. Microplate reader (400 nm)
2. Double-distilled water
3. Normal saline
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Vortex mixer
8. Incubator or water bath

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum and plasma samples can be tested directly.
- **Urine:** Collect fresh urine, then centrifuge at 10,000 × g at 4°C for 10 minutes. Take the supernatant and assay immediately.
- **Tissue Homogenates (1%):** Carefully weigh tissue and add normal saline at a ratio of 9 ml normal saline : 1 g tissue. Homogenize manually using a mechanical homogenizer to prepare a 10% tissue homogenate solution. Centrifuge at 2500 RPM for 10 minutes. Carefully remove the supernatant and dilute 10-fold double-distilled water to prepare a 1% tissue homogenate solution. Assay immediately. Separately determine the protein concentration in the supernatant (abx090644 Bradford Protein Assay Kit is recommended).

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with double-distilled water, then carry out the assay procedure.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Substrate Solution**: Slowly add 30 ml of Reagent A to the Substrate. Mix thoroughly until the powder is fully dissolved. Do not heat. If precipitates are observed, take the supernatant for the assay. Any unused Substrate Solution can be stored at 4°C.
- **0.6 mmol/L Standard Solution:** This is required for serum, plasma, and urine samples. Dilute the 3 mmol/L standard solution 5-fold to prepare the 0.6 mmol/L standard solution.

B. Assay Procedure

Set blank, standard, sample and control tubes. Each sample requires a sample tube and control tube.

1. Serum, plasma and urine samples:

1.1. Add 0.1 ml of double distilled water to each blank tube.

1.2. Add 0.1 ml of **0.6 mmol/L** Standard Solution to each standard tube.

1.3. Add 0.1 ml of sample to each sample tube and each control tube.

Tissue samples:

1.1. Add 0.02 ml of double distilled water to each blank tube.

1.2. Add 0.02 ml of **3 mmol/L** Standard Solution to each standard tube.

1.3. Add 0.02 ml of sample to each sample tube and each control tube.

2. Add 0.5 ml of Reagent A to each blank tube and each standard tube. Mix fully.
3. Add 0.5 ml of Substrate Solution to each sample tube. Mix fully.
4. Incubate at 37°C for 15 minutes.
5. Add 2 ml of Stop Solution to all tubes.
6. Add 0.5 ml of Substrate Solution to each control tube.
7. Add 0.05 ml of Reagent B to all tubes. Mix fully.
8. Zero the spectrophotometer with double distilled water. Measure the absorbance of all tubes at 400 nm using a 1 cm optical path cuvette.

C. Calculation of Results

1. Serum and Saliva samples:

One unit of Beta-N-Acetyl-Glucosaminidase (NAG) activity is defined as the amount of enzyme required in 1 L of sample to produce 1 µmol of p-nitrophenol per minute at 37°C.

$$\mathbf{NAG\ (U/L)} = \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} \times \frac{\mathbf{C_{Standard}}}{\mathbf{t}} \times \mathbf{1000}$$

2. Tissue samples:

One unit of Beta-N-Acetyl-Glucosaminidase (NAG) activity is defined as the amount of enzyme required in 1 g of protein to produce 1 µmol of p-nitrophenol per minute at 37°C.

$$\mathbf{NAG\ (U/g\ protein)} = \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} \times \frac{\mathbf{C_{Standard}}}{\mathbf{C_{Protein} \times t}} \times \mathbf{1000}$$

where:

OD_{Sample}	OD value of sample
$OD_{Control}$	OD value of control
$OD_{Standard}$	OD value of standard
OD_{Blank}	OD value of blank
$C_{Standard}$	Concentration of standard (serum, plasma, urine: 0.6 mmol/L; tissues: 3 mmol/L)
$C_{Protein}$	Concentration of protein in sample (g/L)
t	Reaction time (15 mins)

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.