(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

# β-N-Acetylglucosaminidase (NAG) Activity Assay Kit

Catalog No.: BC00148

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:


✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com


**Shelf life:** Please refer to the label on the outer package.

**Techsupport:** In order to provide you with better service, please inform us the lot number on the label of the outer package.

## Basic Information

| | |
|---|---|
| **Product Name** | β-N-Acetylglucosaminidase (NAG) Activity Assay Kit |
| **Detection Method** | Colorimetric |
| **Sample Type** | Tissues, fluids |
| **Assay Type** | Quantitative |
| **Detection Instrument** | Spectrophotometer (optimal detection wavelength 400 nm) |

## Product Introduction

β-N-acetylglucosaminidase (NAG) is widely found in various tissues, organs, body fluids, red blood cells, white blood cells, and platelets. It is an acidic hydrolytic enzyme in lysosomes.

## Principle

The substrate is hydrolyzed by NAG, releasing free p-nitrophenol. Adding an alkaline solution stops the reaction and causes the p-nitrophenol to develop a color. Enzyme activity units can be calculated by measuring the absorbance at 400 nm.

## Components

| Components | Size | Storage |
|---|---|---|
| Reagent 1 | 40 mL×1 bottle | 2~8℃ |
| Reagent 2 | 1 tube of powder | 2~8℃ |
| Reagent 3 | Terminator 60 mL×2 bottles | 2~8℃ |
| Reagent 4 | 6 mL×1 bottle | 2~8℃ |
| | Crystals may form in cold weather; place in a 37°C water bath until clear before testing. | |

| | | |
|---|---|---|
| Reagent 5 | 3 mmol/L nitrophenol standard stock solution 2 mL × 1 bottle | 2~8℃ |
| | Take a portion of the 3 mmol/L p-nitrophenol standard stock solution and mix it with distilled water at a ratio of 1:4 to prepare a 600 μmol/L p-nitrophenol standard working solution. Prepare the solution immediately before use. | |
| Preparation of substrate buffer: Due to the low solubility of the substrate, when preparing the substrate solution, first mix one vial of reagent two powder with an appropriate amount of reagent one to form a paste. Then, while stirring, gradually add reagent one up to 30 mL, mixing until completely dissolved (do not heat). The prepared substrate buffer is a supersaturated solution. If crystals appear, allow it to stand or centrifuge, and then use the supernatant for detection. Unused substrate buffer can be stored at 4°C for more than two months. | | |

## Storage

The kit has a shelf life of 6 months

## Preparation before the experiment

### Sample processing

1. Preparation: Accurately weigh the sample and add 9 times its volume of physiological saline at a weight (g): volume (mL) ratio of 1:9. Homogenize mechanically under ice-water bath conditions to prepare a 10% homogenate. Centrifuge at 2500 rpm for 10 minutes. Collect the supernatant and dilute it with physiological saline to a 1% homogenate solution for testing. (This homogenate concentration is for reference only; customers can determine the actual required concentration for their sample based on this concentration.)
2. Liquid: direct measurement.

## Operation process

Liquid Sample Handling Sheet:

| Reagents(mL) | Blank tube | Standard tube | Test tube | Control tube |
|---|---|---|---|---|
| Distilled water | 0.1 | -- | -- | -- |
| 0.6 mmol/L standard solution | -- | 0.1 | -- | -- |
| Sample | -- | -- | 0.1 | 0.1 |
| Reagent 1 | 0.5 | 0.5 | | |
| Substrate buffer | | | 0.5 | |
| Mix well and react at 37℃ for 15 minutes. | | | | |
| Reagent 3 | 2 | 2 | 2 | 2 |
| Substrate buffer | -- | -- | -- | 0.5 |
| Reagent 4 | 0.05 | 0.05 | 0.05 | 0.05 |
| Mix well, measure the absorbance value A of each tube at 400 nm with a 1 cm optical path, zero the tube with distilled water. | | | | |

Tissue Sample Operation Table:

| Reagents(mL) | Blank tube | Standard tube | Test tube | Control tube |
|---|---|---|---|---|
| Distilled water | 0.02 | -- | -- | -- |
| 3 mmol/L standard solution | -- | 0.02 | -- | -- |
| Sample | -- | -- | 0.02 | 0.02 |
| Reagent 1 | 0.5 | 0.5 | -- | -- |
| Substrate buffer | -- | -- | 0.5 | -- |
| Mix well and react at 37℃ for 15 minutes. | | | | |
| Reagent 3 | 2 | 2 | 2 | 2 |
| Substrate buffer | -- | -- | -- | 0.5 |
| Reagent 4 | 0.05 | 0.05 | 0.05 | 0.05 |
| Mix well, measure the absorbance value A of each tube at 400 nm with a 1 cm optical path, zero the tube with distilled water. | | | | |

## Result calculation

1、Calculation of NAG enzyme activity in liquid samples:

Unit definition: One unit of enzyme activity (U) is defined as the amount of enzyme that produces 1 μmol of p-nitrophenol per minute when the sample reacts with the substrate at 37°C.

$$\underset{(U/L)}{\text{NAG activity}} = \frac{A_{test} - A_{control}}{A_{Standard} - A_{blank}} \times C_{Standard1} \times \frac{V_{Standard}}{V_{sample}} \div T$$

2、Calculation of NAG enzyme activity in tissue samples:

Unit definition: One unit of enzyme activity (U) is defined as the amount of protein in a gram that reacts with a substrate at 37°C for 1 minute to produce 1 μmol of p-nitrophenol.

$$\underset{(U/gprot)}{\text{NAG activity}} = \frac{A_{test} - A_{control}}{A_{Standard} - A_{blank}} \times C_{Standard2} \times \frac{V_{Standard}}{V_{sample}} \div T \div Cpr$$

**Annotation**

$C_{Standard\ 1}$ : Standard solution concentration , 600 μmol / L;

$C_{Standard\ 2}$ : Standard solution concentration 3 mmol/L (3000 μmol / L ) ;

$V_{standard}$ : Standard sample size, 0.1 $\times 10^{-3}$ L ;

$V_{sample}$: Sample size, 0.1 $\times 10^{-3}$ L ;

T : Reaction time , 15 min ;

Cpr : Protein concentration in tissue homogenate , gprot/L (prot refers to protein);

## Precautions

1. When using a spectrophotometer, the absorbance is linear up to 0.60 or the enzyme activity is linear up to 60 units. If the absorbance is outside this range, the sample should be diluted with physiological saline and retested, and the result multiplied by the dilution factor.
2. Urinary enzyme concentration varies with urine flow rate, therefore urinary enzyme testing requires collecting urine over 24 hours. However, collecting 24-hour urine is inconvenient and inaccurate; therefore, calculating enzyme excretion rate using the ratio of enzyme units to creatinine is more effective.
3. This product is for research purposes only and must not be used for clinical diagnosis. Do not

take it.