beta-N-Acetylglucosaminidase Activity Assay Kit (Colorimetric)

beta-N-Acetylglucosaminidase Activity Assay Kit (Colorimetric) can be used to measure beta-N-Acetylglucosaminidase activity in serum, plasma, urine and cell lysate.

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Package: 100 tests

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INTRODUCTION

Hexosaminidase (EC 3.2.1.52, beta-N-acetylglucosaminidase) is an enzyme involved in the hydrolysis of terminal N-acetyl-D-hexosamine residues in N-acetyl-β-D-hexosaminides.

Even though the alpha and beta subunits of lysosomal hexosaminidase can both cleave GalNAc residues, only the alpha subunit is able to hydrolyze G_{M2} gangliosides because of a key residue, Arg-424, and a loop structure that forms from the amino acid sequence in the alpha subunit. The loop in the alpha subunit, consisting of Gly-280, Ser-281, Glu-282, and Pro-283 which is absent in the beta subunit, serves as an ideal structure for the binding of the G_{M2} activator protein ($G_{M2}AP$), and arginine is essential for binding the N-acetyl-neuraminic acid residue of G_{M2} gangliosides. The G_{M2} activator protein transports G_{M2} gangliosides and presents the lipids to hexosaminidase, so a functional hexosaminidase enzyme is able to hydrolyze G_{M2} gangliosides into G_{M3} gangliosides by removing the N-acetylgalactosamine (GalNAc) residue from G_{M2} gangliosides.

Elevated levels of hexosaminidase in blood and/or urine have been proposed as a biomarker of relapse in the treatment of alcoholism. [Provide by Wikipedia: Beta-N-acetylglucosaminidase]

PRINCIPLE OF THE ASSAY

This beta-N-Acetylglucosaminidase Activity Assay Kit (Colorimetric) is a simple colorimetric assay that measures the activity of beta-N-Acetylglucosaminidase in serum, plasma, urine and cell lysate. This assay is based on the cleavage of p-nitrophenol from a synthetic substrate. *p*-Nitrophenol becomes intensely colored after addition of the stop reagent. The increase in absorbance at O.D. 405 nm after addition of the stop reagent is directly proportional to the enzyme activity.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped at room temperature. Store the Substrate at-20°C and other components at 4°C upon receiving. Shelf life: 12 months after receipt.

Component	Quantity	Storage information
Substrate	10 mL	-20°C
Stop Reagent	12 mL	4°C
Standard (12.5 mM Nitrophenol)	1 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 405 nm
- Centrifuge and centrifuge tube
- Clear flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Substrate and Stop Reagent to samples should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collection the supernatant for assay.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collection the supernatant for assay.

Urine: assayed directly. If particulates are present, centrifuge at 10,000 x g for 3 minutes at 4°C and use the clear supernatant for the assay.

Cell lysate: Collect cells by centrifugation at 2,000 x g for 5 minutes at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold PBS, approximately one million cells per mL. Centrifuge at 14,000 x g for 10 minutes at 4°C. Collection the supernatant for assay.

REAGENT PREPARATION

- **Standard:** Mix 15 µL of Standard (12.5 mM Nitrophenol) with 235 µL of distilled water (final 750 µM). Dilute Standards as follows:

Standard tube	Nitrophenol (µM)	Distilled water (µL)	Standard Premix, 750 µM (µL)
S1	750	0	100
S2	450	40	60
S3	225	70	30
S4	0	100	0

ASSAY PROCEDURE

Equilibrate all components to 37°C. Briefly centrifuge the tubes before opening.

	Standard well	Sample well	Sample Blank well
Each diluted Standard	20 µL		
Each Sample		20 µL	20 µL
Stop Reagent			100 µL
Substrate	80 µL	80 µL	
Tap plate to mix briefly and thoroughly. Incubate for **30 minutes** at **37°C.**			
Stop Solution	100 µL	100 µL	
Tap plate to mix briefly and thoroughly. Read the absorbance at **O.D. 405 nm**.			

CALCULATION OF RESULTS

1. Subtract blank OD (distilled water, S4) from the standard OD values and plot the ΔOD against standard concentrations. Determine the Slope and use the following equation to calculate β-N-Acetylglucosaminidase activity:

   NAG Activity (U/L) = [(OD_{Sample} –$OD_{Sample\ Blank}$) / (Slope x t)] x n

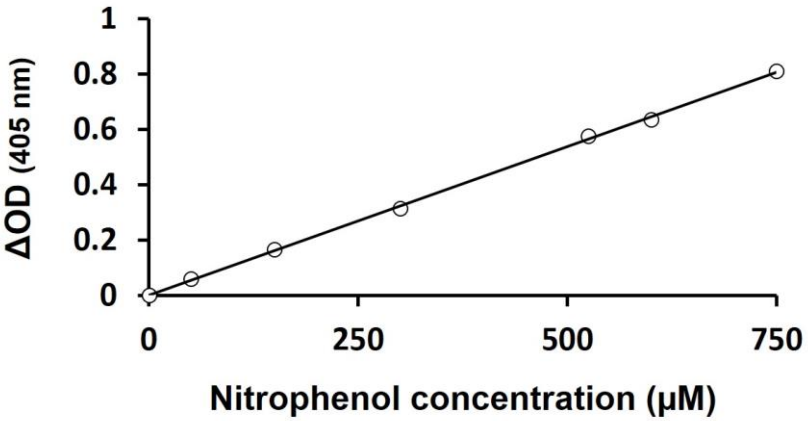
   Note:

   - OD_{Sample} and $OD_{Sample\ Blank}$: the optical density value of the sample and sample blank, respectively.
   - Slope: the slope of the standard curve.
   - t: the reaction time (30 minutes).
   - n: the dilution factor.

2. If sample NAG activity exceeds 50 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with NAG activity < 1 U/L, the incubation time can be extended up to 4 hours for greater sensitivity.
3. Unit definition: 1 Unit (U) will catalyze the conversion of 1 µmole of p-Nitrophenyl N-acetyl-β-D-glucosaminide to p-Nitrophenol and N-acetyl-Dglucosamine per minute at 37°C at pH 4.5.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the beta-N-Acetylglucosaminidase Activity Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.2 U/L