QuantiChrom™ β-N-Acetylglucosaminidase Assay Kit (DNAG-100)

Quantitative Colorimetric β-N-Acetylglucosaminidase Activity Determination

DESCRIPTION

β-N-Acetylglucosaminidase (NAG) is a lysosomal enzyme involved in a variety of biological processes such as the degradation of glycoproteins and glycolipids, cell proliferation, and signal transduction. NAG is found in many tissues in the body, but due to its high molecular weight, it can not be filtered through the glomerular membrane. For this reason, in the presence of tubular damage or a glomerular lesion, urinary NAG activity increases. Elevated NAG levels in urine are an early indication of renal damage, such as injury due to diabetes mellitus, inflammation, nephritic syndrome, urinary tract infection, and more. Various forms of cancer have been associated with increased levels of NAG in serum. Genetically inherited lipid storage disorders, such as Tay-Sachs and Sandhoff disease, arise from deficiencies of the enzyme.

BioAssay Systems' non-radioactive, colorimetric NAG 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 405 nm after addition of the stop reagent is directly proportional to the enzyme activity.

KEY FEATURES

Fast and sensitive. Linear detection range (20 µL sample): 0.2 to 50 U/L for a 30 minute reaction at 37˚C.

High-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

β-N-Acetylglucosaminidase activity determination in biological samples (e.g. urine, serum, plasma, cell lysate, etc.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Substrate: 10 mL **Stop Reagent:** 12 mL
Standard: 1 mL (12.5mM Nitrophenol)

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

Precautions: 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.

PROCEDURES

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.

Sample Preparation: Serum and plasma can be assayed directly. For urine samples containing precipitation, centrifuge at 10,000 x g, 4°C for 3 minutes and assay the supernatant.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min 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 min at 4°C. Remove supernatant for assay.

Reagent Preparation: Equilibrate all components to 37°C. Briefly vortex or pipette up and down all components to ensure fresh reconstitution.

Standard Preparation: Mix 15 µL of 12.5 mM Nitrophenol standard with 235 µL dH_2O to make 750 µM Premix.

No	Premix + dH_2O	Vol (µL)	Nitrophenol (µM)
1	100 µL + 0 µL	100	750
2	60 µL + 40 µL	100	450
3	30 µL + 70 µL	100	225
4	0 µL + 100 µL	100	0

Reaction Preparation:

1. Transfer 20 µL of each sample into two separate wells (OD_{SAMPLE} and $OD_{SAMPLE\ BLANK}$). Transfer 20 µL of each standard (OD_{STD}) into separate wells of a clear flat bottom 96-well plate.
2. Add 100 µL of stop reagent to the *Sample Blank* wells.
3. Add 80 µL of the substrate solution to all standard, sample, and sample blank wells. Tap plate briefly to mix.
4. Incubate at 37°C or desired temperature for 30 minutes. Add 100 µL of Stop Reagent to each standard and sample well. *Note: Do not add anything more to the sample blank wells.* Tap plate briefly to mix.
5. Read OD_{405nm}.

CALCULATION

Subtract blank OD (water, #4) 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.

$$\text{NAG Activity} = \frac{OD_{SAMPLE} - OD_{SAMPLE\ BLANK}}{t \bullet \text{Slope}} \times n \quad (U/L)$$

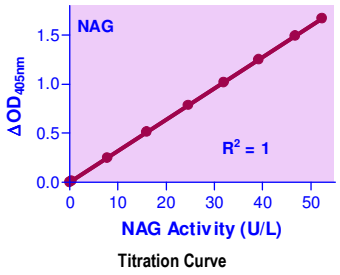
where OD_{SAMPLE} is the OD_{405nm} value for each sample and $OD_{SAMPLE\ BLANK}$ is the OD_{405nm} value of the sample blank. Slope is the slope of the linear regression fit of the standard points and *t* is the reaction time (30 min). *n* is the dilution factor.

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-D-glucosamine per min at 37°C at pH 4.5.

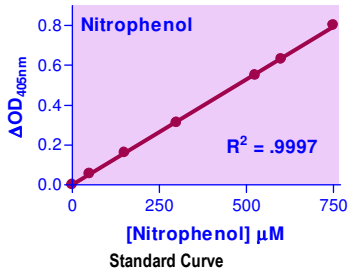
Note: If sample NAG activity exceeds 50 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with NAG activity < 1 U/L, the incubation time can be extended up to 4 hours for greater sensitivity.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes, and plate reader.



Titration Curve



Standard Curve

LITERATURE

1. Sheira, G., et al. (2015). Urinary biomarker N-acetyl- β-D-glucosaminidase can predict severity of renal damage in diabetic nephropahy. Journal of Diabetes &Metabolic Disorders. 12; 14:4.
2. Severini, G., et al. (1995). A study of serum glycosidases in cancer. J Cancer Res Clin Oncol. 121(1):61-3.
3. Hartmann, D., et al. (2015). Plasma N-acetyl-glucosaminidase in advanced gastro-intestinal adenocarcinoma correlates with age, stage and outcome. Future Oncology. 11(2):193-203.

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