Technical Bulletin

β-N-Acetylglucosaminidase Assay Kit

Catalog Number MAK456

Product 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 cannot be filtered through the glomerular membrane. 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, or urinary tract infection. 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.

The β-N-Acetylglucosaminidase Assay Kit 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 the addition of stop reagent is directly proportional to the enzyme activity. The linear detection range of the assay method is 0.2 to 50 U/L for a 30 minute reaction.

The kit is suitable for β-N-Acetylglucosaminidase activity determination in urine, serum, plasma, cell lysate, and other biological samples.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Substrate 10 mL
  Catalog Number MAK456A
- Stop Reagent 12 mL
  Catalog Number MAK456B
- Standard (12.5 mM Nitrophenol) 1 mL
  Catalog Number MAK456C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Microcentrifuge capable of RCF ≥ 10,000 × *g*
- 1.5 mL microcentrifuge tubes
- Phosphate Buffered Saline (PBS) (Catalog Number P3813 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store the Substrate at -20 °C and all other components at 2-8 °C upon receiving.

Preparation Instructions

Equilibrate all components to 37 °C prior to use. Briefly vortex each component or pipette up and down to ensure a homogenous solution.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Serum and plasma

Serum and plasma samples can be assayed directly.

Urine

For urine samples containing precipitation, centrifuge at 10,000 × *g*, 4 °C for 3 minutes. Retain the supernatant for assay.

Cell Lysate

1. Collect cells by centrifugation at 2,000 × *g* for 5 minutes at 4 °C.
2. For adherent cells, do not harvest cells using proteolytic enzymes. Instead, use a rubber policeman or cell scraper.
3. Homogenize or sonicate cells in an appropriate volume of cold PBS at a concentration of approximately one million cells per mL.
4. Centrifuge at 10,000 × *g* for 15 minutes at 4 °C.
5. Remove supernatant and retain for assay.

Standard Curve Preparation

1. Prepare a 750 µM Nitrophenol Standard by mixing 15 µL of the 12.5 mM Nitrophenol Standard with 235 µL of purified water.
2. Prepare Nitrophenol Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Nitrophenol Standards

Well	750 µM Nitrophenol Standard	Purified Water	Nitrophenol (µM)
1	100 µL	-	750
2	60 µL	40 µL	450
3	30 µL	70 µL	225
4	-	100 µL	0

Reaction Preparation

Note: This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to Samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

1. Transfer 20 µL of each Sample into two separate wells of a clear flat bottom 96-well plate for use as Sample and Sample Blank (OD_S and OD_{SB}).
2. Transfer 20 µL of each Standard (OD_{STD}) into separate wells.
3. Add 100 µL of Stop Reagent into each Sample Blank well.
4. Add 80 µL of Substrate solution to all Standard, Sample, and Sample Blank wells. Tap plate briefly to mix.
5. Incubate the plate at 37 °C or desired temperature for 30 minutes.
6. Add 100 µL of Stop Reagent to each Standard and Sample well. Do not add additional Stop Reagent to Sample Blank wells as it was added in Step 3. Tap plate briefly to mix.

Measurement

Read optical density (OD) at 405 nm.

Results

1. Subtract the OD_{Blank} (Standard #4) reading from the remaining Standard OD readings. Plot the corrected Standard OD readings against the Standard concentrations.
2. Determine the slope of the Standard curve using linear regression.
3. Calculate the β-N-Acetylglucosaminidase concentration of the sample:

β-N-Acetylglucosaminidase (U/L) =

$$\frac{OD_S - OD_{SB}}{T \times Slope} \; x \; DF$$

where

OD_S = Optical density at 405 nm of Sample

OD_{SB} = Optical density at 405 nm of the corresponding Sample Blank

T = Reaction time (The procedure standard is 30 minutes but may be extended for low or shortened for high activity Samples)

Slope = Slope of the Standard curve

DF = Sample Dilution Factor (DF = 1 for undiluted Samples)

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

Unit definition: 1 Unit (U) of β-N-Acetyl-glucosaminidase will catalyze the conversion of 1 μmole of p-Nitrophenyl N-acetyl-β-D-glucosaminide to p-Nitrophenol and N-acetyl-D-glucosamine per minute at 37 °C at pH 4.5.

Figure 1.
Typical Nitrophenol Standard Curve

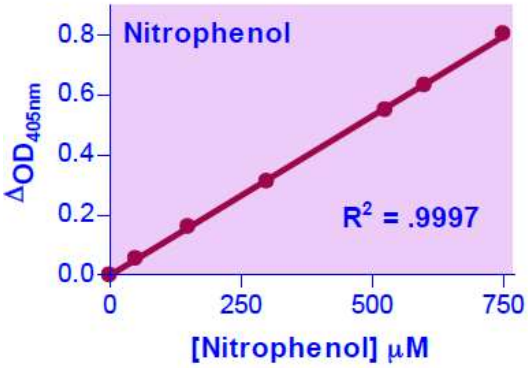
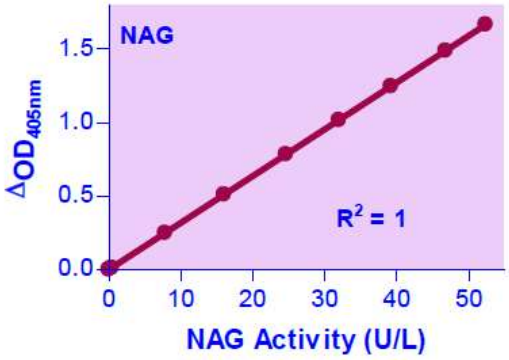


Figure 2.
Typical β-N-Acetylglucosaminidase Titration Curve



References

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2. Severini, G., et al., A study of serum glycosidases in cancer. *J. Cancer Res. Clin. Oncol.*, **121**, 61-3 (1995).
3. Hartmann, D., et al., Plasma N-acetyl-glucosaminidase in advanced gastro-intestinal adenocarcinoma correlates with age, stage and outcome. *Future Oncol.*, **11**, 193-203 (2015).

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