Technical Bulletin

β-N-Acetylglucosaminidase Assay Kit

Catalogue Number CS0780

Product Description

β-N-Acetylglucosaminidase (NAG, N-acetyl-β-D-glucosaminidase) is a lysosomal enzyme in mammalian cells, which takes part in intracellular degradation of glycolipids and glycoproteins.[1] High activities of this enzyme have been detected in human kidney, lung, and liver lysosomes. In humans, different genetically determined deficiencies of the enzyme result in lipid storage disorders.[2,3] Moreover, elevated levels of serum NAG are associated with certain forms of cancer,[4] and the increase in total NAG activity in the urine is associated with renal disorder.[5]

The assay is based on the hydrolysis of the NAG substrate, 4-Nitrophenyl N-acetyl-β-D-glucosaminide (NP-GlcNAc), by the enzyme. This enzymatic hydrolysis of the substrate releases *p*-nitrophenol, which upon ionization in basic pH, can be measured colorimetrically at 405 nm.

The β-N-Acetylglucosaminidase Assay Kit provides all the reagents required for efficient detection of β-N acetylglucosaminidase activity in cell lysates, tissue homogenates, lysosomal isolated fractions, or purified enzyme preparations.

The kit was tested on CHO, HeLa, HEK 239T, A431, HepG2, U937, and Jurkat cells, and on rat brain, spleen, kidney, and liver tissues.

Components

The kit is sufficient for fifty 1 mL reactions or 500 multiwell plate reactions of 100 µL.

- Dilution Buffer — 8 mL
  Catalogue Number D2318
- 4-Nitrophenyl N-acetyl-β-D-glucosaminide — 50 mg
  Catalogue Number N9376
- Citrate Buffer Solution, 0.09 M — 100 mL
  Catalogue Number A4855
- *p*-Nitrophenol Standard Solution, 10 mM — 1 mL
  Catalogue Number N7660
- β-N-Acetylglucosaminidase from Jack beans — 1 vial
  Catalogue Number M0545
- Sodium Carbonate — 5 g
  Catalogue Number S2127

Equipment Required but Not Provided

- Spectrophotometer or ELISA reader (405 nm)
- Cuvettes (3 mL, Catalogue Number C5291), or 96-well plates (flat bottom, Catalogue Number P7366)
- 37 °C water bath

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Use ultrapure water (17 MΩ·cm or equivalent) for preparation of reagents.

Substrate Solution (1 mg/mL) - Dissolve 10 mg of the 4-Nitrophenyl N-acetyl-β-D-glucosaminide (NP-GlcNAc, Catalogue Number N9376) in 10 mL of the 0.09 M Citrate Buffer Solution (Catalogue Number A4855). Mix the Substrate Solution with rocking/shaking on a horizontal shaker at room temperature.

> **Note**: The substrate does **not** easily dissolve in the buffer. It may take ~1 hour of shaking to completely dissolve the substrate. Use of a larger container (50 mL) may aid dissolution.

The Substrate Solution should be stored on ice during the experiment. For longer storage of at least one month, store at –20 °C.

NAG Control Enzyme - Just before use, dilute a portion of the β-N-Acetylglucosaminidase (NAG, Catalogue Number M0545) 1:100 in the Dilution Buffer (Catalogue Number D2318). The enzyme is supplied as an ammonium sulfate suspension, therefore, vortex the tube vigorously and pipette up and down a few times to ensure a uniform suspension before diluting the enzyme. Store the NAG Control Enzyme on ice. **Do not freeze**.

> **Note**: If the signal obtained with the NAG Control Enzyme is too high/low, adjust the dilution accordingly.

Stop Solution (sodium carbonate solution) - Add 118 mL of ultrapure water to the sodium carbonate bottle (Catalogue Number S2127) and mix well with a magnetic stirrer until completely dissolved. Store the Stop Solution at room temperature.

Standard Solution - Before performing the assay in 96-well plates, dilute 5 µL of the 10 mM *p*-Nitrophenol Standard Solution (Catalogue Number N7660) with 995 µL of Stop Solution. Vortex briefly and store on ice.

NAG Sample – The use of the CelLytic™ M (Catalogue Number C2978) or CelLytic™ MT (Catalogue Number C3228) Cell Lysis reagents is recommended for cell or tissue sample preparation, respectively.

Storage/Stability

The kit is shipped on wet ice and storage at 2–8 °C is recommended. Upon arrival it is recommended to store the NP-GlcNAc (Catalogue Number N9376) at -20 °C and the Sodium Carbonate (Catalogue Number S2127) at room temperature.

Procedure

The NAG enzyme hydrolyzes the NP-GlcNAc substrate under acidic conditions (pH ~4.7) at 37 °C. The enzymatic hydrolysis of the substrate releases *p*-nitrophenol.

$$\text{NP-GlcNAc} + H_2O \xrightarrow{\text{NAG}} p\text{-Nitrophenol} + \text{GlcNAc}$$

Addition of the basic Stop Solution causes ionization of the *p*-nitrophenol to form the yellow *p*-nitrophenylate ion. The absorbance of the *p*-nitrophenylate ion is measured at 405 nm.[6,7]

Assay in Tubes (3 mL final volume)

1. Equilibrate the Substrate Solution to 37 °C by incubating for several minutes.
2. Set the spectrophotometer at 405 nm.
3. Add the reaction components to the tubes according to Table 1 and vortex the tubes briefly.

Table 1.
Reaction Scheme for Tube Assays

	Substrate Solution	**Sample**
Blank*	1 mL	----
Positive Control	995 µL	5 µL of NAG Control Enzyme
Test Sample	900-990 µL	10-100 µL of sample

* A blank reaction (Substrate Solution without enzyme) should be run, since a portion of the NP-GlcNAc may hydrolyze spontaneously during the incubation time.

Note: If required (the absorbance is too low), the volume of the Sample/NAG Control Enzyme may be as large as 10% of the reaction volume.

4. Incubate the tubes for 5-30 minutes at 37 °C. The incubation time required for the Test Sample(s) depends on the enzyme concentration. The incubation time required for the Positive Control is usually 5-10 minutes.
5. Stop the reaction by adding 2 mL of Stop Solution. Vortex briefly and transfer the reaction mixture to a cuvette. The reaction mixture should be yellow in color.
6. Use the blank reaction mixture to zero the spectrophotometer and then read the absorbance of the samples.

Note: Read the Test Samples immediately after the incubation, within 30 minutes.

96-Well Plate Assay

It is recommended to perform the assays in triplicate.

1. Equilibrate the Substrate Solution and the Standard Solution to 37 °C by incubating for several minutes.
2. Set the plate reader at 405 nm.
3. Add the reaction components to the 96-well plates according to Table 2 and mix using a horizontal shaker or by pipetting.

Table 2.
Reaction Scheme for 96-Well Plate Assays

	Substrate Solution	Sample	Standard Solution
Blank*	100 µL	-	-
Standard	-	-	300 µL
Positive control**	98 µL	2 µL of NAG Control Enzyme	-
Test Sample	90-99 µL	1-10 µL of sample	-

* A blank reaction (Substrate Solution without enzyme) should be run, since a portion of the NP-GlcNAc may hydrolyze spontaneously during the incubation time.

** If required (the absorbance is too low), the volume of the enzyme can be increased up to 10% (10 µL) of the total reaction volume.

4. Incubate the plate for 5-10 minutes at 37 °C. If you suspect that the NAG activity of the Test Sample is low, the incubation time can be extended up to 30 minutes.
5. Stop the reactions by adding 200 µl of Stop Solution to each well, except to wells containing the Standard Solution.
6. Measure the absorption at 405 nm.

Results

Activity Calculation for Cuvette Assay

$$\text{Units/mL} = \frac{A_S \times 3 \times DF}{18.3 \times T \times V}$$

where:

A_S = Absorbance of the Test Sample at 405 nm

3 = Final volume in milliliters in the reaction cuvette after addition of stop solution

DF = Enzyme dilution factor (DF = 1 for undiluted samples)

18.3 = Millimolar extinction coefficient for *p*-nitrophenolate ion

T = Reaction time in minutes

V = Volume of the Test Sample in milliliters

Activity Calculation for 96-Well Plate Assay

$$\text{Units/mL} = \frac{(A_S - A_B) \times 0.05 \times 0.5 \times DF}{A_{STD} \times T \times V}$$

where:

A_S = Absorbance of the Test Sample at 405 nm

A_B = Absorbance of the Blank at 405 nm

0.05 = µmole/mL of *p*-nitrophenol in the Standard Solution

0.3 = Final volume in milliliters of the 96-well plate reaction after addition of the stop solution

DF = Enzyme dilution factor

A_{STD} = Absorbance of the Standard Solution at 405 nm

T = Reaction time in minutes

V = Volume of the Test Sample in milliliters

Unit definition: 1 unit will hydrolyze 1.0 µmole of 4-Nitrophenyl N-acetyl-β-D-glucosaminide to *p*-nitrophenol and N-acetyl-β-D-glucosaminide per 1 minute at pH 4.7 at 37 °C.

References

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cs0780pis Rev 09/22