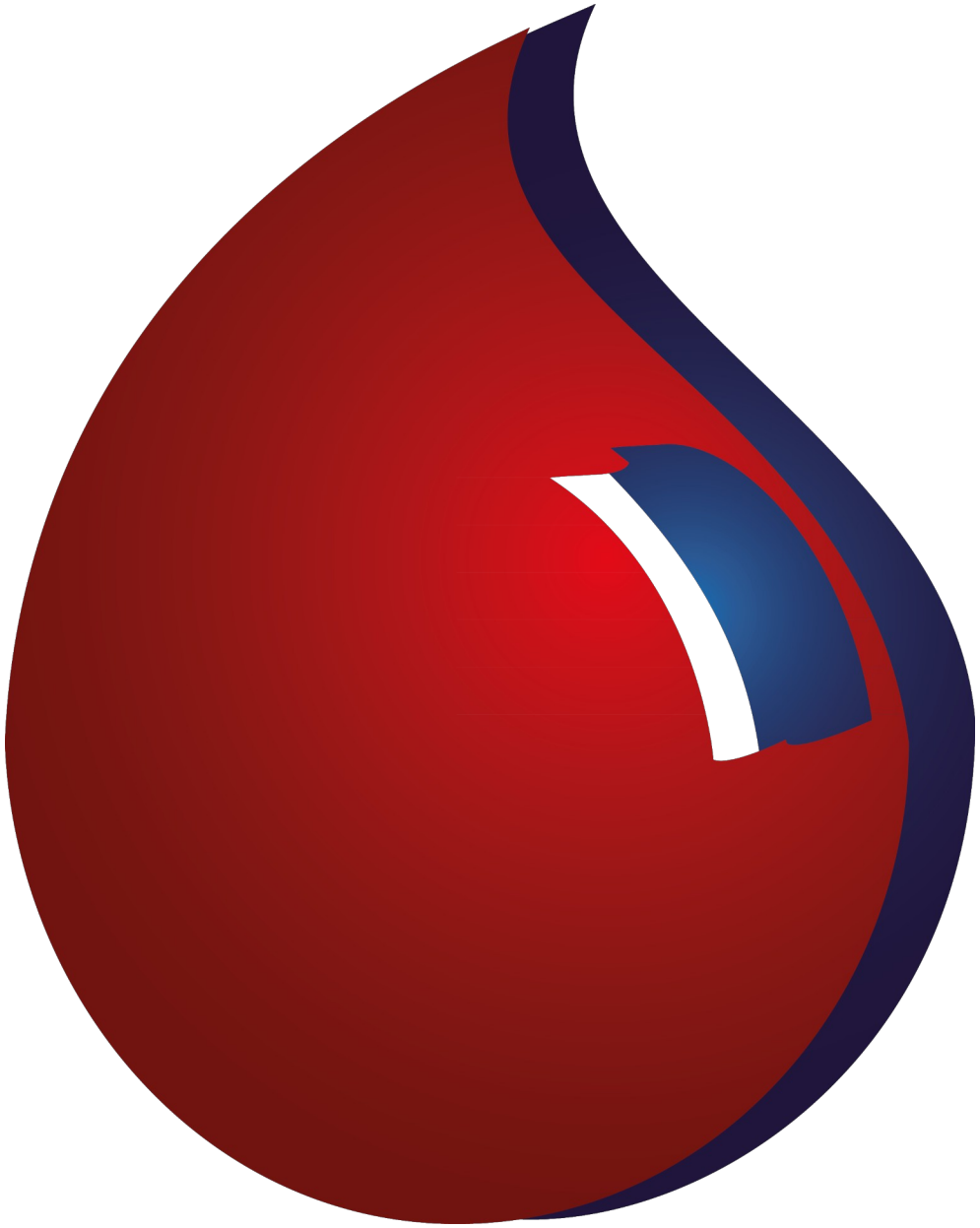

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N-Acetyl-glucosaminidase (NAG) Assay

Kit Manual

www.HaemoScan.com

Product number: K006
Version: 02-11-2026

Introduction

N-acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme of the renal proximal tubular cells that is released into the urine following injury to the tubular system of the kidney. Such injury may occur after renal ischaemia or may be induced by an inflammatory reaction. NAG is determined by means of a substrate conversion assay: under appropriate conditions, NAG present in a urine sample converts a chromogenic substrate. After colour development, the optical density is measured. The NAG activity (U/L) is quantified using calibrators with known enzyme activities. A quantitative measure of tubular damage can be obtained by simultaneously measuring urinary creatinine to correct for urine dilution.

Principle of the Test

This procedure describes the determination of NAG in urine samples. The samples are incubated with a chromogenic substrate at acidic pH for one hour, after which an alkaline Stop solution is added and the optical density is measured at 400 nm. Because samples may contain components that cause a high background signal, a sample blank is determined for every sample, in which the Stop solution is added before, instead of after, the substrate.
The NAG activity is expressed in units per litre (U/L). One unit converts 1 µmol of substrate per minute at pH 4.25 and 25 °C.

Precautions

- Store the kit at −20 °C or colder, as cold as possible. The shelf life of one year is based on storage at −20 °C.
- The kit is intended for research use only. It is not intended for diagnostic or therapeutic procedures.
- Do not use the kit beyond its expiry date.
- Do not combine reagents from kits with different lot numbers.
- The Controls are prepared from human urine that has not been tested for pathogens. Handle the Controls and all urine samples as potentially infectious material (biosafety level 2). Some components contain protein of bovine origin.
- The Stop solution causes serious eye irritation. Wear eye protection; in case of contact with the eyes, rinse cautiously with water for several minutes and seek medical advice if irritation persists.
- Dispose of the kit components, samples and contaminated materials in accordance with local and national regulations. For further information, please refer to the Safety Data Sheet (SDS), available at www.haemoscan.com.
- Wear disposable nitrile gloves, a laboratory coat and safety glasses.
- Avoid contact of reagents with skin and mucous membranes.

- Use disposable pipette tips throughout the procedure to avoid contamination of reagents.

Contents of the Kit

• NAG substrate	22 mL	1 bottle
• Stop solution	22 mL	1 bottle
• NAG standard (25 U/L)	500 µL	1 tube
• Dilution buffer	4 mL	1 bottle
• Control 1 (normal), human urine	500 µL	1 tube
• Control 2 (high), human urine	500 µL	1 tube

Additional Materials and Equipment

The following materials and equipment are required but are not provided with the kit:

- Calibrated adjustable pipettes with disposable tips
- Microcentrifuge tubes
- Incubator at 37 °C
- Transparent 96-well flat-bottom microplate
- Plate shaker
- Microplate reader capable of measuring at 400 nm

Test Procedure

Reagent Preparation

- **Substrate:** Warm to 37 °C before use.
- **Calibrators:** Prepare a two-fold serial dilution of the NAG standard in Dilution buffer in separate tubes, according to Table 1. Use these dilutions as the calibration curve.

Table 1. Preparation of NAG calibrators.

		NAG activity (U/L)
CAL1	100 µL NAG standard + 100 µL Dilution buffer	12.5
CAL2	100 µL CAL1 + 100 µL Dilution buffer	6.25
CAL3	100 µL CAL2 + 100 µL Dilution buffer	3.13
CAL4	100 µL CAL3 + 100 µL Dilution buffer	1.56
CAL5	100 µL CAL4 + 100 µL Dilution buffer	0.78
CAL6	100 µL CAL5 + 100 µL Dilution buffer	0.39
CAL7	Dilution buffer	0

Assay Procedure

1. Pipette 100 µL of Stop solution into all blank wells of a transparent 96-well flat-bottom microplate (see the example plate layout in Table 2).
2. Pipette 20 µL of each calibrator, control and sample into the appropriate wells.
3. Warm the plate for 5 minutes at 37 °C.
4. Add 100 µL of pre-warmed NAG substrate to each well.
5. Shake the plate for 20 seconds on a plate shaker.
6. Incubate the plate for 60 minutes at 37 °C.
7. Add 100 µL of Stop solution to each well except the blank wells (see Table 2).
8. Shake the plate for 20 seconds on a plate shaker.
9. Measure the optical density at 400 nm.

Table 2. Suggested 96-well plate layout for the NAG assay. Each control and sample is measured in one well and its sample blank in the well directly below it.

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CTRL1	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
B	CAL2	CTRL1 Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank
C	CAL3	CTRL2	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
D	CAL4	CTRL2 Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank
E	CAL5	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
F	CAL6	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank
G	CAL7	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
H	CAL7	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank	Sample Blank

Calculations

1. Subtract the mean optical density of CAL7 (0 U/L) from all optical density values.
2. Construct a calibration curve ($y = a \cdot x + b$) from the calibrators.
3. Calculate the NAG activity of the blank wells, the controls and the samples by interpolation on the calibration curve.
4. Subtract the value of each blank well from that of the corresponding control or sample.

Assay Criteria

- The correlation coefficient of the calibration curve should be ≥ 0.98.

Reference Values

In a group of healthy volunteers (n = 8), a mean urinary NAG activity of 1.04 U/L was found (SD 0.40 U/L; range 0.4–1.7 U/L).

Example Results

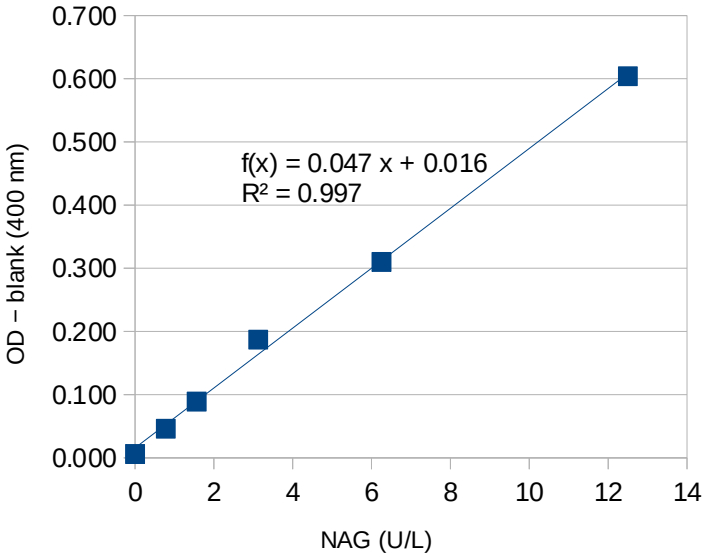

Figure 1. Example of a NAG calibration curve.