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N-acetyl-glucosaminidase Assay

Kit Insert

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Introduction

N-acetyl-glucoseaminidase (NAG) is excreted into the urine following injury to the tubular system of the kidney. This injury may occur after renal ischemia 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 development of the color the optical density is determined. The concentration (U/L) is quantified by applying standards with known concentrations of the enzyme. A quantitative measure of tubular damage can be obtained after simultaneous measurement of urea, to correct for dilution.

Principle of the Test

This standard operating procedure describes the determination of NAG in urine samples. The samples are incubated with a chromogenic substrate. After one hour of incubation at low pH, a basic stop solution is added and the optical density at 400 nm is measured. Because the samples may contain components which can result in a high background signal, for every sample a sample blank value is determined in which the stop solution is added prior to the reaction, instead of after the reaction.
The concentration of NAG is expressed in units/liter (U/L). One unit converts 1 mmol of substrate per minute at a pH of 4.25 and a temperature of 25°C.

Precautions

- Keep the kit in an as cold as possible freezer. The shelf life of one year is based on -20 ºC.
- The kit is intended for research use only.
- The kit should not be used beyond its expiry date.
- Do not combine reagents from kits with different lot numbers.
- Chemicals and reagents have to be treated as hazardous waste according to biohazard safety guidelines or regulations.
- Wear disposable (latex) gloves when handling specimens and reagents.
- Never pipette by mouth and avoid contact of skin and mucous membranes
- Use disposable pipette tips throughout the procedure to avoid contamination of reagents.

Contents of the Kit

- NAG Substrate 11 mL 1 bottle
- Stop Solution 11 mL 1 bottle
- NAG Standard (25 U/L) 500 µL 1 tube
- Dilution Buffer 2 mL 1 bottle
- Control 1, normal 250 µL 1 tube
- Control 2, high 250 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.
- (Micro-centrifuge) tubes.
- Incubator at 37 °C.
- Transparent 96-well flat-bottom microplate
- Plate shaker.
- Spectrophotometer capable of measuring at 400 nm.

Test Procedure

Reagent Preparation

- **Substrate:** warm to 37°C before use.
- **Calibrators:** The NAG standard is used stepwise (1:1) diluted with Dilution Buffer in separate vials. Use these dilutions as standard curve (Table 1).

Table 1. Preparation of NAG Calibrators.

		NAG Concentration (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 Stop Solution in all Blank wells of a transparent 96-well flat-bottom microplate (please refer to the example plate layout [Table 2]).
2. Pipette 20 µL of calibrators, controls and samples
3. Warm the plate at 37°C for 5 minutes
4. Add 100 µL prewarmed Substrate to each well
5. Shake the plate for 20 seconds on a plate shaker
6. Incubate the plate at 37°C for 60 minutes
7. Add 100 µL Stop Solution to each well except the Blank wells (please refer to the example plate layout [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 template for the NAG Assay

	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 Blank	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 the 'CAL7 Blank' (NAG concentration = 0 U/L) from all the optical density values
2. Construct a calibration curve ($y=a \cdot x+b$) with the calibrators. The NAG concentration should go on the y-axis and the OD values of the calibrators on the x-axis.
3. Calculate the NAG concentration in the Blank wells, the control samples and the other samples by means of interpolation on the calibration curve.
4. Subtract the values of the Blank wells from the corresponding samples

Assay Criteria

- The correlation coefficient of the calibration curve should be ≥0,98.

Reference values

In a group (n=8) of healthy volunteers, an average NAG concentration of 1.04 U/l (SD = 0.40 U/l, Range: 0.4 – 1.7 U/l) was found.

Characteristics

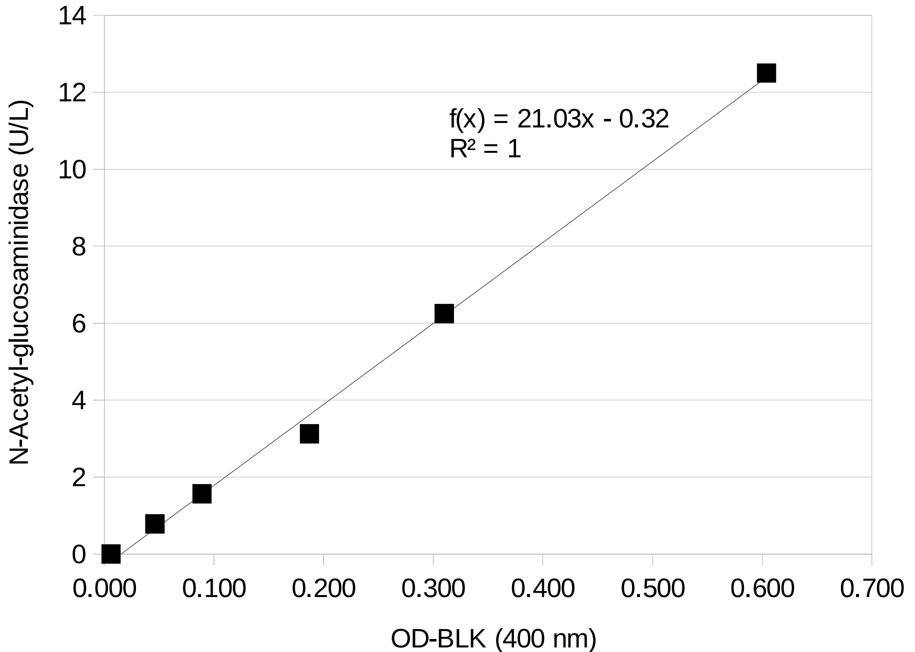

Figure 1. Example of a NAG calibration curve.