N-acetyl-β-D-glucosaminidase (NAG)

Method: Colorimetry

Cat .No.	Size	Instrument
GB330S	R1: 1×60 ml R2: 1×20 ml	For Hitachi 717 &S himadzuCL7200/8000
GS331S	R1: 1×60 ml R2: 1×20 ml	For Hitachi917 & OlympusAU640/400/600

INTENDED USE

For the *in vitro* quantitative determination of NAG activity in urine.

CLINICAL SIGNIFICANCE

NAG is a lysosomal enzyme involved in the breakdown metabolism of glycoproteins. Increased NAG levels in urine are an early indication of renal disease and can serve as a valuable renal monitoring test in disorders such as nephritic syndrome, glomerulunephritis, drug abuse associated nephrotoxicity, diabetes-associated nephropathy, hypertension and urinary tract infections.

ASSAY PRINCIPLE

NAG hydrolyses ammonium 5-[4-(2- acetamido-2-deoxy-beta-D-glucopyranosyloxy)-3- methoxy-phenylmethylene]-2-thioxothiazolidin-4-one-3-ethanoate (VRA-GlcNAc), the product formation is detected by development of color at 505 nm upon addition of alkaline buffer.

REAGENT COMPOSITION

Contents	Concentration of Solutions
Reagent 1	
Citrate acid buffer	pH 4.8
VRA-GlcNAc	1 mmol/L
Stabilizer	
Reagent 2	
Na_2CO_3 buffer	pH 9.5
Stabilizer	

SAMPLE COLLECTION AND PREPARATION

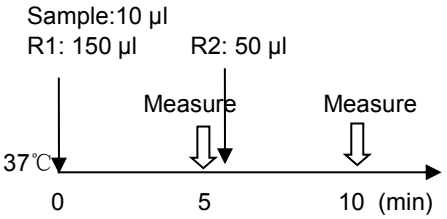
Fresh urine samples should be used when possible. However, urine samples can be stored for one week at 2-8℃ or up to 1 month at -20℃ without significantly affecting NAG activity. Samples containing low amount of preservative can be used (less than 0.02% sodium azide). NAG activity is pH-sensitive, hence urine samples should have a pH range between 4.0-8.0.

STABILITY AND PREPARATION OF REAGENTS

All reagents are ready to use.
Stable up to the expiry date when stored at 2-8℃.
The reagent after opening is stable for 28 days on-board the analyser .

ASSAY PROCEDURE

Test Procedure for Analyzers (HITACHI 917)
Assay Mode: 2 point end
Wave Length (main/sub): 505 nm/700 nm



1. Mix 10 µl sample with 150 µl R1 and incubate at 37℃ for 5 minutes, then read initial absorbance A_1.
2. Add 50 µl R2 into cuvette, mix and incubate for 5 minutes at 37℃, read final absorbance A_2.
3. Calculate the absorbance change $\Delta A=A_2-A_1$.

CALCULATION

$$\text{NAG (U/L)} = \frac{\Delta A_{sample} - \Delta A_{blank}}{\Delta A_{calibrator} - \Delta A_{blank}} \times \text{Calibrator value}$$

QUALITY CONTROL

For quality control, use NAG Control as daily quality control sera and can be purchased separately. Values should fall within a specific range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check reaction temperature.
3. Check expiration date of kit and contents.

NORMAL VALUE

Urine: 0.3 - 12 U/L
It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

SPECIFIC PERFORMANCE CHARACTERISTICS

LINEARITY

The method is linear up to 300 U/L. Sample above this concentration should be diluted with 0.9% NaCl and reassay. Multiply the result by dilution factor.

PRECISION

The CV of the test should be less than 5%.

Intra assay precision	
N=20	Level
Mean (U/L)	40.8
SD	0.23
CV	0.55%
Inter assay precision	
N=5	Level
Mean(U/L)	40.9
SD	0.35
CV	0.84%

SENSITIVITY

The minimum detectable level that can be distinguished from zero has been determined as 0.29 U/L.

INTERFERENCE

The following analyze were tested up to the levels indicated and found not to interfere:
Bilirubin: 15 mg/dl
Ascorbic Acid: 60 mg/dl

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BSA: 100 mg/dl
Urea: 6 g/dl

CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:
Y=1.5655+1.3542, and a correlation coefficient of 0.999.
70 patient samples were analyzed .

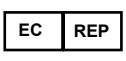
SAFETY PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handing laboratory reagents.
2. Solution R1a contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
3. Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.
4. All specimens used in this test should be considered potentially infectious. Universal Precautions, as they apply at your facility, should be used for handling and disposing of materials during and after testing.

REFERENCES

1. Krishna KS， Kirubakaran MG， Pandey AP， et al.Urinary NAG and AAP in the diagnosis of graft rejection after live donor renal transplantation. Clin Chim Acta，1985，150（2）:69-85.
2. IIstran pocsl et.al. VRA-GlcNAc: novel substrate for N-Acetyl-β-D-glucosaminidase applied to assay of this enzyme in urine.Clinical chemisty, 1990,36(11): 1884-1888

INDEX OF SYMBOLS

	Manufacture
	Catalogue Number
	Lot number
	Date of manufacture
	Use by(Expiration date)
	For In-Vitro Diagnostic use only
	Stored at 2-8℃
	Attention:See instruction for use
	Authorized Representative in the European Company

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