GENTAUR

DATA SHEET

N-acetyl-β-D-Glucosaminidase (NAG) Assay
Catalog Number: BQ062A-EAKP

Intended Use
The N-acetyl-β-D-glucosaminidase (NAG) assay kit is for determination of NAG in patient urine samples. The assay is for investigational use or export only.

Background
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
The reagents of the assay kit are in stable liquid formulation that allows ease of use coupled with enhanced performance characteristics. NAG hydrolyses 2-methoxy-4-(2'nitrovinyl)-phenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (MNP-GlcNAc) to 2-methoxy-4-(2'-nitrovinyl)-phenol product. The product formation is detected by development of color at 505nm upon addition of an alkaline buffer.

Specimen Collection and Handling
Fresh urine samples should be used when possible. However, urine samples can be stored for one week at 2-8 ^{o}C or up to 1 month at -20 °C 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.

Reagent Composition

Reagent 1 (R1)	1 x 75 mL	MNP-G1cNAc, HCI
Reagent 2 (R2)	1 x 15 mL	Citric acid, Potassium phosphate (pH 4.7)
Reagent 3 (R3)	1 x 30 mL	Sodium carbonate buffer (pH 10)
Calibrator *	1 vial	Reconstitute with 2 mL dH_2O to make standard at concentration indicated on vial label.
Control Set* (purchased separately)	2 vials	Reconstitute with 2 mL dH_2O to make standard at concentration indicated on vial label.

* After reconstitution, leave calibrator and control at 2-8 ^{o}C for 24 hours to equilibrate.

Warnings
1. For Research Use Only in the USA. Not for use in diagnostic procedures.
2. Specimens and reagents containing human sourced materials should be handled as if potentially infectious, using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Avoid swallowing and contact with skin or mucous membranes.

Materials Required But Not Provided
An analyzer capable of dispensing 2 reagents and of measuring absorbance at 505 nm with temperature control (37 ^{o}C).

Reagent Preparation
Reagent 1 and Reagent 2 should be mixed in a volume ratio of 5:1 (5 volumes of R1 and 1 volume of R2) to make the R1+R2 solution mix. Each test requires 750µL of the solution mix. The solution mix, thus prepared, is stable for 1 week when stored capped at 2-8°C. Reagents are light sensitive. Reagents from different lots must not be interchanged. Reconstituted calibrator and controls should be equilibrated at 2 – 8 °C for 24 hours prior to use.

Reagent Stability and Storage
The Reagent 1 – Reagent 2 solution is stable for 1 week when stored capped at 2 – 8 °C. Reagent 3 is stable until the expiration dated indicated on its label when unopened and stored at 2 – 8 °C. Once opened, reagents are stable for 1 month at 2 – 8 °C in original bottles, if closed tightly after use. Once reconstituted, the calibrator and controls are stable for two weeks.

Assay Procedure
See attached program parameters for COBAS and Hitachi systems. All reagents should be equilibrated at room temperature prior to use.

1. For a manual method, pipette reagents in glass tubes (12 x 75mm) in the order shown below. It is important to adhere to a timed schedule. For example, add samples 30 seconds apart.
2. Incubate at 37°C for 5 minutes and add 250µL of Reagent 3 to each reaction for color development.
3. Transfer to cuvette for immediate absorbance readings at 505nm.
4. Calculate ΔOD 505nm for samples and calibrator by subtracting the blank value.

Order of addition	Reagent blank	Standard	Samples
dH_2O	50µL	-	-
NAG Calibrator	-	50µL	-
Urine Sample	-	-	50µL
Reagent Solution mix	750µL	750µL	750µL

Sample (NAG, IU/L) =

$$\frac{\text{Sample OD 505nm} - \text{Blank OD 505 nm}}{\text{Standard OD 505nm} - \text{Blank OD 505nm}} \times \text{Standard Units}$$

Calibration
A single calibrator is needed for running the assay in calibration mode. NAG activity in sample is determined from linear calibration curve using the included standard. Daily calibration is recommended.

Quality Control
Good laboratory practice recommends the use of control materials. Users should follow the appropriate federal, state and local guideline concerning the running of external quality control.

To ensure adequate quality control, normal and abnormal control with known values should be run as unknown samples.

Results
NAG results are printed out in IU/L.

Reference Range
Literature reports cite subjects having a NAG activity in the range of 0.3 -12 IU/L. There is no apparent significant difference in NAG excretion between males and females. NAG activity is known to vary with age and diuresis, hence a NAG index (ratio of NAG activity to urinary creatinine) is often used to minimize variability. [2]

Limitations
If a sample has higher than 200 IU/L of NAG, then it should be diluted 1:2 or 1:5 with dH_2O prior to measurement.

Interferences
No significant interference from hemoglobin or albumin. Interference from bilirubin occurs only at levels higher than 5 mg/L.

References
1. Price, R.G. & Whiting, P.H., Urinary Enzymes (1992), 203-221. Eds: Jung, K, Matteheimer and Burchardt H. Springer-Verlag, Berlin
2. Yuen CT et al, Clin Chem Acta (1982) 124: 195-204

Cobas Mira Parameters
Temperature 37ºC

User the following parameters with calibrator for calibration.

Measurement Mode	Absorb
Reaction Mode	R-S-SR1
Calibration Mode	Slope Avg
Reagent Blank	Reag/DIL
Cleaner	No
Wavelength	500 nm
Decimal position	3
Unit	U/L
Sample Cycle	1
Sample volume	10.0 uL
Sample dilution	H_20
Dilution Volume	0.0 uL
Reagent cycle	1
Reagent 1 (solution mix) volume	150 µL
Dilution volume	0.0 µL
Start R1 (reagent 3) cycle	13
Reagent volume	50 µL
Dilution volume	0.0 µL
Sample Limit	No
Reagent direction	Increase
Convers. Factor	1.0000
Offset	0.0000
Test range low	0.000 U/L
Test range High	200.00 U/L
Number of steps	1
Calc. Step A	Endpoint
Readings first	12
Readings last	13
Calibration	
Cali. Interval	Each day
Time	No
Blank	
Reagent range low	0.0
High	3.5
Blank range low	-0.04
High	3.5
Standard pos	1
Standard-1	*

* Entered By Operator

Each cycle is 25 seconds on the Cobas Mira S analyzer.

The above reagent parameters has **not been fully validated** for this analyzer. The parameters are based on BQ Kits's knowledge of the analyzer and reagents, and should perform adequately. However, you should use these parameters as guidelines in conjunction with your Quality Control Program for validation.

Hitachi 717 Parameters
Temperature 37ºC

Use the following parameters with calibrator for calibration.

Test	NAG
Assay Code	2 Pinot
Assay Point	(24)-(26)
Wavelength	800/505
Calibration Method	Linear
Unit	U/L
Sample volume	(10)(10)
Reagent vol. R1 (mix solution)	(150)(100)(NO)
Reagent vol. R3	(50)(100)(NO)
STD (1) CONC.-POS	(0)-(1)
STD (2) CONC.-POS	(*)-(2)
ABS.Limit	32000-Increase
Expected value (normal Value)	0.3-12
Tech Limit	0-200

* Entered By Operator
Hitachi 717: Each cycle 12 second

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Olympus AU400 Parameters
Temperature 37 ºC

Use the following parameters with calibrator for calibration.

General
Test Name: NAG Type: Urine Operation: Yes
Sample Volume 10µL Dilution 0 µL Pr-Dilution Rate 1
Reagents: Min OD Max OD
R1 volume 150 µL Dilution 0 µL L:H:
R2 volume 50 µL Dilution 0 µL
Wavelength: Pri. 520 Sec. 800 Reagent OD Limit
Method: End First L:-2.000; First H: 2.500
Reaction Slope: +Last L: -2.000; Last H: 2.500
Measuring Point 1: First 9; Last 11 Dynamic Range:
Measuring Point 2: First ; Last L: O H: 200.0
Linearity Correlation Factor:
No-Lag-Time: A: 1.0000 B: 0.000
Onboard stability Period 999
Calibration Type AB Formula: Y=AX+B
Counts 2 Process CONC
Cal No. OD CONC Factor/OD-L Factor/OD-H
Point 1*.* -999999.0 999999.0
Point 2
Point 3
Point 4
Point 5
Point 6
Point 7
Advanced Calibration: No
Calibration Stability Period: 999

. Input by Operator

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