NAG

Colorimetric Determination
of N-Acetil-β-Glucosaminidase
in Urine and in Serum

4 x 10 Tests **REF CY08-40T**

Available for quality control:

1 x 1 ml AAP NAG STANDARD **REF 7506**

PRINCIPLE

N-acetyl-β-glucosaminidase (NAG) catalyzes the hydrolysis of p-nitro-phenil-N-acetyl-β-D-glucosaminide to p-nitrophenol and N-acetylgluco-samine. The liberated p-nitrophenol is proportional to the enzymatic activity and is colorimetric defined in an alkaline medium.

REAGENTS

Kit components:	REF CY08-40T	Quantity
REAGENT 1 (lyo) Substrate	**CY08-40TR1**	**4 vials**
REAGENT 2 Buffer pH 5.2	**CY08-40TR2**	**2x15 ml**
(*) **REAGENT 3** Alkalizer	**CY08-40TR3**	**2x17 ml**

STABILITY: stored at 2-8°C to keep reagents stable up to the expiration date on the label.

PREPARATION OF REAGENT 1

REF 7114

Reconstitute the contents of a vial of Reagent 1 with exactly 5.2 ml of distilled water.

Shake gently until complete dissolution.

STABILITY: 3 days at 2-8°C.

SAMPLE

Non dialyzed urine.
STABILITY: 2 days at 2-8°C, 2 months at -20°C.
Serum: dilute the serum 1:4 with saline solution.
STABILITY: non diluted serum: 10 days at 2-8°C, 2 months at -20°C.

MANUAL ASSAY PROCEDURE

Wavelength:	spectrophotometer 405 nm photometer 400-420 nm
Optical path:	1 cm
Reading:	against distilled water
Temperature:	37°C
Reaction time:	15 minutes
Linearity:	urine : up to 60 U/L serum: up to 240 U/L

Pipette into test tubes or cuvettes labeled as it follows:
S: sample, B/S: blank sample, B/R: blank reagent:

	S	B/S	B/R
Reagent 1	0.5 ml	---	0.5 ml
Reagent 2	---	0.5 ml	---

After reagents reach 37°C, add mixing accurately:

Sample	0.1 ml	0.1 ml	---
Saline solution	---	---	0.1 ml

Incubate for exactly 15 minutes at 37°C and add mixing accurately:

Reagent 3	0.400 ml	0.400 ml	0.400 ml

Within 30 minutes, read the sample (As), the blank sample (Abs) and the blank reagent (Abr) absorbencies against distilled water.

CALCULATION

URINE:
Activity in U/L = (As - Abs - Abr) x 36
Activity per mg of creatinine in mU/mg of creatinine =
(U/L x 100) / mg of creatinine for 100 ml

SERUM:

Activity in U/L = (As - Abs - Abr) x 144

REFERENCE VALUES

URINE:

Concentration: 5.7 ± 1.9 U/L
4.2 ± 1.2 mU/mg of creatinine

Excretion rate:
male 4.3 ± 1.3 mU/min
female 3.9 ± 1.3 mU/min

SERUM:

Concentration: 26 ± 3,1 U/L

QUALITY CONTROL

All Clinical Chemistry laboratories should implement a quality control program. Control Urine of human origin are available for this purpose on request:

PERFORMANCE CHARACTERISTICS

Linearity: up to 60 U/L in urine and to 240 U/L in serum.
For higher values, dilute 1 volume of sample with 9 volumes of saline solution, repeat the test and multiply the result by 10.

Within-run precision (urine sample):

	Level 1	Level 2
Average (U/L)	5.01	35.1
DS	0.043	0.407
CV %	0.86	1.16

Between-run precision (urine sample):

	Level 1	Level 2
Average (U/L)	5.50	32.5
DS	0.085	0.828
CV %	1.54	2.55

Correlation: FAR NAG kit shows a correlation coefficient of 0.98 in comparison to another kit available on the market.

DISPOSAL

The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS

Reagent 3 may cause severe eye irritation (H319). It also cause skin irritation (H315). In case of skin contact: rinse thoroughly with water. In case of contact with the eyes: rinse thoroughly for several minutes. If eye irritation continues, call a doctor.

NOTES

1. (*) dangerous reagents are marked by an asterisk. Refer to safety data sheet.
2. Centrifuge turbid samples at 3000 rpm x g for about 5 minutes.
3. One blank reagent is enough for each assay series; if obtained values are constant, the determination can be done periodically .
4. Urine pH and protease presence have a great influence on urinary enzyme stability. NAG is more stable with pH between 5 and 7. It is recommended to adjust the pH of highly acid or alkaline urines.
5. Each laboratory should define its own reference values.
6. Reaction volumes can be proportionally changed.
7. Disposal waste according to local laws.
8. Chemistry analyzer parameters are available.

REFERENCES

1. Maruhn D., Clin. Chim. Acta 73 (1976) 453
2. Gressner A. M., Roebruck P., Clin. Chim. Acta 124 (1982) 315

MANUFACTURER

FAR
Via Fermi, 12 - 37026 Pescantina - VERONA - ITALY
tel +39 045 6700870
website http://www.fardiag.com
e-mail: order@fardiag.com
e-mail: fardiag@fardiag.com

KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalog number
	temperature limits
	use by
	caution
	consult accompanying documents

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