

AMMONIA

Enzymatic UV Determination of Ammonia in Plasma

22 x 2.5 ml

REF CY04-55

PRINCIPLE

In presence of glutamate dehydrogenase enzyme (GLDH), ammonia reacts with con l'-ketoglutarate and NADPH to form glutammate and NADP+. The absorbance decrease of NAPDH at 340 nm, due to its oxidation, defines the plasmatic ammonia concentration in the sample.

REAGENTS

Kit composition:	REF CY04-55	Quantity
REAGENT 1/A (Iyo)	CY04-55R1A	22 vials
Tris buffer pH=8.6 NADPH		
REAGENT 1/B	CY04-55R1B	2 x 35 ml
Triethanolamine pH=8.6 ADP		
REAGENT 2 (pre-dosed)	CY04-55R2	2 vials
GLDH		

WARNING: close the vials immediately after use. The ammonia present in the air might be absorbed by liquids.

STABILITY: stored at 2-8°C, reagents are stable up to expiration date.

PREPARATION OF WORKINGS REAGENTS

PREPARATION OF REAGENT 1 (1/A+1/B)

Reconstitute the contents of a vial of Reagent 1/A with 2.5 ml of Reagent 1/B. Close the vial and shake accurately until complete dissolution.

STABILITY: 24 hours at 20-25°C.

PREPARATION OF REAGENT 2 (2+1/B)

Add exactly 0.5 ml of Reagent 1/B to a vial of Reagent 2. Close the vial and leave the solution at room temperature for 10 minutes, shaking lightly until complete dissolution.

Leave to stand for 10 minutes before use.

STABILITY: 3 weeks at 2-8°C; 5 days at a 20-25°C.

SAMPLE

Plasma.

Draw blood from a stasis-free vein and centrifuge in a closed centrifuge tube as soon as possible, within maximum 15 minutes after collection.

Perform the assay on plasma as soon as possible.

EDTA can be used as anticoagulant.

STABILITY: 2 hours maximum at 2-8°C.

MANUAL ASSAY PROCEDURE

Wavelength:	340 nm
Optical path::	1 cm
Reading:	against air
Temperature:	20-25°C
Method:	fixed time
Linearity:	up to 700 µg/dl (412 µmol/L)
Sample/Reagents:	1/5

Let reagents and samples reach room temperature before use.

Prepare a blank reagents for each assay series.

Pipette in test tube or cuvettes labeled as follows:

	B/R	S
Plasma	----	0.5 ml
Reagent 1	2.5 ml	2.5 ml

Mix carefully and incubate for 10 minutes at the test temperature. Pour into cuvette and read absorbancies of blank reagent (Abr1) and sample (As1) at 340 nm against air.

Then add:

	B/R	S
Reagent 2	0.02 ml	0.02 ml

Mix carefully and incubate for 10 minutes at the test temperature. Pour into cuvette and read absorbancies of blank reagent (Abr2) and sample (As2) at 340 nm against air.

Then add again:

	B/R	S
Reagent 2	0.02 ml	0.02 ml

Mix carefully and incubate for 10 minutes at the test temperature. Pour into

cuvette and read absorbancies of blank reagent (Abr3) and sample (As3) at 340 nm against air.

Define ΔA of Blank (ΔABR) and of Sample (ΔAS):

$$\Delta ABR = (Abr1 - Abr2) - (Abr2 - Abr3)$$

$$\Delta AS = (As1 - As2) - (As2 - As3)$$

CALCULATION

$$\text{Ammonia } (\mu\text{g/dL}): [\Delta AS - (\Delta ABR \times 0.83)] \times 1633$$

$$\text{Ammonia } (\mu\text{mol/L}): [\Delta AS - (\Delta ABR \times 0.83)] \times 959$$

REFERENCE VALUES

men: 25-94 µg/dL (14.7-55.3 µmol/L)

women: 19-82 µg/dL (11.2-48.2 µmol/L)

Each laboratory should define its own reference values.

PERFORMANCE CHARACTERISTICS

Linearity: up to 700 µg/dL (412 µmol/L).

For higher values, dilute the sample 1:5 with ammonia-free water, repeat the test and multiply the result by 5.

Within-precision:

	Level 1	Level 2
Average [µg/dL]	30	250
DS	0.205	3.6
CV %	0.68	1.44

Between-precision:

	Level 1	Level 2
Average [µg/dL]	45	288
DS	0.526	8.5
CV %	1.17	2.95

Correlation:

FAR Ammonia kit shows a correlation coefficient equal to 0.98 in comparison to another kit available on the market.

NOTES

- (*) dangerous reagents are marked by an asterisk. Refer to safety data sheet.
- wear proper protective clothes - do not pipette by mouth
- disposal residual reagents and waste according to local laws
- read the kit instructions before performing the test
- use only the reagents contained in the kit and recommended reagents
- do not use reagents of different lots
- do not use reagents that have expired
- do not use reagents from other manufacturers
- chemistry analyzer parameters are available.

DISPOSAL

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

WARNINGS AND PRECAUTIONS

The reagents may contain non-reactive components and various preservatives. Contact with the skin and ingestion should be avoided. Use the normal precautions expected with correct behaviour in laboratory.

REFERENCES

1. H.A.M. Jacobs and F.M.F.G.– *Clin.Chim.Acta*, Vol. 43, (1973), 81-86;

MANUFACTURER

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KEY SYMBOLS

	In Vitro diagnostic medical device
	batch number
	catalogue number
	temperature limits
	use by
	caution
	consult accompanying documents