ALA

Chromatographic – Colorimetric Determination
of δ–Aminolevulinic Acid
in Urine

40 tests **REF 3603**

INTENDED USE

Kit for quantitative *in vitro* determination of δ-Aminolevulinic Acid in urine.

PRINCIPLE

In hemoglobin biosynthesis, lead inhibits the activity of ALA dehydrase enzyme, which catalyzes the condensation of two ALA (δ-aminolevulinic acid) molecules to form a PBG (porphobilinogen) molecule. In lead intoxication, only a small part of ALA is involved in the hemoglobin synthesis, as the main part is emptied in urine. Hence the increase of urinary ALA is the most reliable index to evaluate the occurred lead poisoning.
In the screening of lead exposed workers, it has been customary to contemporary define urinary PBG and ALA. In accordance with recent studies, PBG determination lost its diagnostic value because it has been ascertained that PBG concentration in lead exposed workers is very low, or even equal to zero like in normal subjects not exposed to lead. Consequently, urinary ALA can be effectively defined without any preventive PBG removal by passage on a column of anionic resin. Only one cationic resin column is used, on which the ALA is adsorbed. After washing of interfering substances, it is eluted and quantitatively determined by Ehrlich reaction.

REAGENTS AND COLUMNS

Kit components:	**REF 3603**
REAGENT 1 Sodium acetate	**2 x 225 ml**
***REAGENT 2** Acetylacetone	**1 x 3 ml**
***REAGENT 3/A** DMAB (powder)	**1 vial**
***REAGENT 3/B** Acetic acid	**1 x 50 ml**
***REAGENT 3/C** Perchloric acid	**1 x 10 ml**
REAGENT 4 Standard δ-aminolevulinic acid 0.2 g/L	**1 x 1 ml**
COLUMNS chromatographic columns	**40**

(*) Dangerous reagents are marked by an asterisk. Refer to MSDS.

STABILITY: stored at 2-8°C, sealed reagents and columns are stable up to the expiration date on the label.

EQUIPMENT REQUIRED BUT NOT SUPPLIED

Bain-marie 100°C.
Spectrophotometer or filter photometer at 553 nm (520 - 570 nm).

PREPARATION OF WORKING REAGENTS

REAGENT 3 (3/A + 3/B)
Dissolve the contents of a vial of Reagent 3/A into the vial of Reagent 3/B and shake until complete dissolution.
STABILITY: 6 months at 2-8°C.

EHRLICH REAGENT (3/C + 3)
Add 1.9 ml of Reagent 3/C to 10 ml of Reagent 3 and shake to obtain a homogeneous solution. This solution thus prepared will be enough for 5 assays. If required, higher quantities can be prepared considering each column needs 1 ml of this reagent.
STABILITY: **6 hours at room temperature.**

SAMPLE

24 hour urine.
Collect the urine and add concentrated hydrochloric acid until the related pH is lower than 6.
Mix the urine, measure the volume and store at 2-8°C.
STABILITY: at least one month stored at 2-8°C and at pH < 6.

MANUAL ASSAY PROCEDURE

Wavelength:	553 nm (520 - 570 nm)
Optical path:	1 cm
Reading:	against blank reagent
Temperature:	boiling bain-marie
Method:	colorimetric endpoint
Linearity:	up to 6 mg/100 ml
Sensitivity:	0.1 mg/100 ml
C.V. (intra-assay):	2%
C.V. (inter-assay):	3%

PREPARATION OF THE COLUMN

Take the upper cap off and snap the bottom tip off. Let the liquid completely flow out and discard it.

CHROMATOGRAPHIC SEPARATION

Pipette into the column:

Distilled water	10.0 ml	discard the eluate
Urine	1.0 ml	discard the eluate
Distilled water	20.0 ml	discard the eluate

Place the column over a clean test tube and pipette:

Reagent 1	10.0 ml	collect the ALA ELUATE

COLORIMETRIC REACTION

Accurately mix the collected eluate and pipette into 3 test tubes labeled as it follows:

	Blank reagent	Sample	Standard
ALA ELUATE	---	2.0 ml	---
Reagent 4 standard	---	---	0.02 ml
Reagent 1	2.0 ml	---	1.98 ml
Reagent 2	0.04 ml	0.04 ml	0.04 ml

Shake vigorously and incubate the test tubes in a hot bain-marie for 10 minutes. Cool under running water, mix well and pipette into 3 new test tubes:

Pre incubated solution	1.0 ml	1.0 ml	1.0 ml
Ehrlich reagent	1.0 ml	1.0 ml	1.0 ml

Mix and incubate at room temperature for 15 minutes. Read the sample (As) and the standard (Astd) absorbencies against the blank reagent, preferably within 5-10 minutes. The developed color reaches its highest intensity after 15 minutes and remains stable for 15 minutes.

CALCULATION

δ-aminolevulinic acid (mg/100 ml) = (A sample /A standard) x 2
mg ALA/100 ml x 10 x L of 24 hour urine = mg ALA / 24 hours

REFERENCE VALUES

δ-amino levulinic acid: up to 0.60 mg/100 ml

NOTES

1. Indication of lead intoxication degree:

ALA (mg/100 ml)	Intoxication Degree
up to 0.60	None
0.60 - 1.50	Moderate
1.50 - 3.00	High
3.00 - 6.00	Very high
more than 6.00	Critical

2. The reagent quantities are enough to perform 56 tests (40 samples, 8 stardards and 8 blanks).
3. FAR kit (y) to define ALA shows a correlation coefficient of 0.989, in comparison to a direct method.

REFERENCE

1. K. Tomokuni, M. Ichiba, Y. Hirai et T. Hasegawa, Clin. Chem. , 33 (9), 1665-1667 (1987)

IVD CE

Issue 01 - Sep 2006 RR

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