ALA – PBG

Chromatographic – Colorimetric Determination
of δ-Aminolevulinic Acid and Porphobilinogen
in Urine

20 tests **REF 3605**

INTENDED USE

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

PRINCIPLE

Urine is made pass through a 2-column system. On the first column, which contains an anioninc resin, porphobilinogen (PBG) and other interfering substances are adsorbed. On the second, which contains a cationic resin, δ-aminolevulinic acid (ALA) is adsorbed; then it is eluted and quantitatively dosed by Ehrlich reaction. PBG in the first column can be selectively eluted and quantitatively dosed by Ehrlich reaction.

REAGENTS AND COLUMNS

Kit components:	REF 3605
REAGENT 1 Sodium acetate	**1 x 225 ml**
***REAGENT 2** Solvent	**1 x 2 ml**
***REAGENT 3/A** Cromogenic agent (powder)	**1 vial**
***REAGENT 3/B** Glacial acetic acid	**1 x 100 ml**
***REAGENT 3/C** Perchloric acid	**1 x 20 ml**
REAGENT 4 Standard δ-aminolevulinic acid 0.2 g/L	**1 x 2 ml**
REAGENT 5 Acetic acid 1	**1 x 90 ml**
COLUMNS chromatographic columns for ALA + PBG	**20 + 20**

(*) 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 REAGENTS

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

EHRLICH'S 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. The solution thus prepared will be enough for 11 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 urine and add concentrated hydrochloric acid until related pH is lower than 6. Mix, measure the volume and store at 2-8°C.
STABILITY: δ-amino levulinic acid is stable for at least one month; PBG for at least 24 hours, if 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:	hot bain-marie
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 COLUMNS

Use an ALA column and a PBG column for each sample. Take upper cap off and snap bottom tip off. Let the liquid completely flow out.
Place the PBG column over the ALA column, to let PBG eluate drop into ALA column. Pipette into the upper column (PBG):

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

Remove the upper column which will be used to define PBG and store it protected from light.

ALA DETERMINATION

Place ALA column over a clean test tube and pipette:

Reagent 1	10.0 ml	collect the ALA eluate

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 within 15 minutes and remains stable for 15 minutes.

PBG DETERMINATION

Place the PBG column over a clean test tube and pipette:

Reagent 5	2.0 ml	collect the eluate

Let the liquid completely flow out, then add:

Reagent 5	2.0 ml	collect the eluate

At the end of the procedure, only one PBG eluate of 4 ml volume is obtained. Mix well and pipette into 2 different test tubes:

	Blank reagent	Sample
PBG eluate	---	1.0 ml
Distilled water	1.0 ml	---
Ehrlich reagent	1.0 ml	1.0 ml

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

CALCULATION

δ-amino levulinic acid (ALA) mg/100 ml = (As/Astd) x 2
mg ALA/100 ml x 10 x L of 24 hour urine = mg ALA/24 hours
Porphobilinogen (PBG) mg/100 ml = A sample x 2.92
mg PBG/100 ml x 10 x L of 24 hour urine = mg PBG/24 hours

REFERENCE VALUES

δ-aminolevulinic acid: up to 0.60 mg/100 ml
Porphobilinogen: up to 0.15 mg/100 ml

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

NOTE

FAR kit (y) to define ALA-PBG shows a correlation coefficient of 0.989, in comparison to a direct method.

REFERENCE

1. J.R. Davis et S.L. Andelman Arch. Environ Health" 15,53-59 (1967)

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