# ALA – PBG

Chromatographic – colorimetric determination of δ-Aminolevulinic Acid and Porphobilinogen on urine

**10 + 10 tests** **REF KR02-10**

## INTENDED USE

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

## PRINCIPLE

Let urine pass through a 2-column system. Porphobilinogen (PBG) and other interfering substances are adsorbed on the first column which contains an anioninc resin. δ-aminolevulinic acid (ALA) is adsorbed on the second column which contains a cationic resin; 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 KR02-10 |
|---|---|
| **REAGENT 1** Sodium acetate | **1 x 60 mL** |
| ***REAGENT 2** Solvent | **1 x 1 mL** |
| **REAGENT 3/A** Cromogenic agent (powder) | **1 vial** |
| ***REAGENT 3/B** Glacial 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** |
| **REAGENT 5** Acetic acid 1 M | **1 x 22 mL** |
| **COLUMNS** chromatographic columns for ALA + PBG | **10 + 10** |

(*) 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.

## REQUIRED BUT NOT PROVIDED

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. This solution is enough for 11 assays. If required, bigger 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 pH is lower than 6. Mix well, 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. Remove cap and break bottom tip. Let the liquid flow out completely.
Place the PBG column over the ALA column and let PBG eluate drop into ALA column.
Pipette into the upper column (PBG):

| | | |
|---|---|---|
| Distilled water | 1.0 mL | discard the eluate |
| Urine | 0.5 mL | discard the eluate |
| Distilled water | 1.0 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 on a clean tube and pipette:

| | | |
|---|---|---|
| Distilled water | 5.0 mL | discard the eluate |
| Reagent 1 | 5.0 mL | collect the ALA eluate |

Pipette into 3 tubes labeled as 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 tubes in a hot bain-marie for 10 minutes.
Cool under running water, mix well and pipette into 3 new 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) absorbances against the blank reagent, 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 on a clean tube and pipette:

| | | |
|---|---|---|
| Distilled water | 5.0 mL | discard the eluate |
| Reagent 5 | 1.0 mL | collect the eluate |

Let the liquid flow out completely, then add:

| | | |
|---|---|---|
| Reagent 5 | 1.0 ml | collect the eluate |

At the end of the procedure, only one PBG eluate of 2 ml volume is obtained. Mix well and pipette into 2 different 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, 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 0.98, in comparison to a direct method.

## DISPOSAL

The product must be used for professional assay only. Dispose of the product according to national/international laws.

## REFERENCE

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

## KEY SYMBOLS

| | |
|---|---|
| IVD | In Vitro diagnostic medical device |
| LOT | batch number |
| REF | catalogue number |
| | temperature limits |
| | use by |
| | caution |
| | read instructions for use |

IVD CE

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