U/C PORPHYRINS

Chromatographic - Spectrophotometric Determination
of Total Porphyrins
and Separation of Uro-Porphyrins and Copro-Porphyrins
in Urine

20 tests **REF KR11-20**

INTENDED USE

Kit for quantitative *in vitro* determination of Total Porphyrins in urine.

PRINCIPLE

Porphyrins are adsorbed on two chromatographic columns containing anionic resin. After interfering substances are washed away, uro- and copro-porphirins are eluted from the first column. After copro-porphyrins are selectively washed away by acetate buffer, uro-porphyrins are eluted from the second column. Porphyrins are spectrophotometrical dosed and the concentration is defined by Allen formula or a fluorometer.

REAGENTS AND MATERIALS

Kit components:	REF KR11-20
***REAGENT 1** Hydrochloric acid	**1 x 105 ml**
***REAGENT 2** Acetate buffer	**1 x 105 ml**
COLUMNS Chromatographic columns	**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.

ADDITIONAL REAGENTS NOT INCLUDED IN THE KIT

Sodium carbonate for analysis. Glacial acetic acid.

EQUIPMENT BUT NOT SUPPLIED

Spectrophotometer: suitable to clearly select the three wavelengths as per Allen formula for quantitative dosage.
Filter fluorometer: excitation 405 nm
emission 595 nm (590-600 nm).

SAMPLE

24 hour urine. Store the samples protected from light.

DETERMINATION OF THE PREFORMED PORPHYRINS

Perform the test immediately after urine collection, as porphyrinogens transformation into porphyrins may give misleading results. Porphyrinogens at 6-9 pH completely transform after 36 hours.

DETERMINATION OF TOTAL PORPHYRINS
(preformed + porphyrinogens)

Prepare the sample in one of the following ways:

1. Measure the volume of the collected urine, take a sample and add sodium carbonate to obtain a 1% (w/v) solution.
Store the sample at room temperature, protected from light.
Perform the test after 24 hours.

STABILITY: the sample is stable for one week.

2. To obtain total porphyrins immediately, bring urine to 5.0 pH with glacial acetic acid and incubate in a hot bain-marie for 30 minutes, away from light.

MANUAL ASSAY PROCEDURE

Wavelength:	380, 400-407, 430 nm
Linearity:	up to 13 mg/L
Light path:	1 cm
Sensitivity: Total porphyrins	colorimetry 40 µg/L fluorometry 10 µg/L
Sensitivity: Coproporphyrins	colorimetry 150 µg/L fluorometry 20 µg/L
Reading:	against Reagent 1
Temperature:	room temperature
C.V. (intra-assay):	2 %
C.V. (inter-assay):	5%

PREPARATION OF THE COLUMN

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

CHROMATOGRAPHIC SEPARATION

Pipette into two columns:

	Column 1 (copro + uro)	Column 2 (uro)	
Urine	1.0 ml	1.0 ml	discard the eluate
Reagent 2	---	10.0 ml	discard the eluate
Distilled water	5.0 ml	1.0 ml	discard the eluate

Place the columns over a clean test-tube and pipette:

Reagent 1	2.5 ml	2.5 ml	collect the eluate

Let the liquid completely flow, then add 2.5 ml Reagent 1 again and collect the eluate together with the previous one. Mix eluates (5 ml) and read absorbencies at 380 nm, with maximum absorption between 400 and 407 nm and at 430 nm against Reagent 1 (Allen's correction).
WARNING: If sodium carbonate was added to the sample to bring it to alkaline pH, when adding Reagent 1 or 2 some CO_2 bubbles may form, slowing the liquid flow in the column. Eliminate bubbles by lightly inclining the column.

CALCULATION

Calculate the difference between absorbance values (ΔA) measured according to the following formula:
ΔA = 2A (400 - 407 nm) - [A (380 nm) + A (430 nm)]

COLUMN 1
Total porphirins (µg/L) = ΔA x 3857
µg total porphyrins/L x L /24h urine = µg total porphyrins/24 hours

COLUMN 2
Uroporphirins (µg/l) = ΔA x 4266
µg uroporphyrins/L x L of 24h urine = µg uroporphyrins/24 hours
Coproporphirins (µg/L)=total porphyrins (µg/L) - uroporphyrins (µg/L)
µg coproporphyrins/L x L /24h urine = µg coproporphyrins/24 hours

REFERENCE VALUES

Total porphyrins:	< 220	µg/24 hours
Coproporphyrins:	35 - 150	µg/24 hours
Uroporphyrins:	15 - 50	µg/24 hours

NOTES

1. Urines preserved with sodium carbonate can not be used to define ALA or PBG, as they are not stable at a basic pH.
2. To define only porphyrins, use only one column for each sample and follow the procedure described for column 1.
3. For porphyrins measurerement in a normal values range, it is advisable to use a fluorometer. Prepare a standard coproporphyrin (approximate concentration of 1 µg/ml) and, for dilution, a working standard of 40 µg/L (0.2 ml standard mother solution + 4.8 ml Reagent 1). Read the adsorbencies at 380 nm, 401- 402 nm and 430 nm and define the concentration as it follows:
   Standard (µg/ml)=
   = 747.6 x [2A (401 - 402 nm) - A (380 nm) - A (430 nm)].
   Read the 405/595 nm fluorescence of eluates (F1) and (F2), of the working standard (Fst) and the Reagent 1 (Fo) and calculate as it follows:
   µg total porphyrins/24 hours=
   = [(F1 - Fo)/(Fst - Fo)] x 5.3 x standard (µg/ml) x L of 24h urine
   µg uroporphyrins/24 hours=
   = [(F2 - Fo)/(Fst - Fo)] x 6.3 x standard (µg/ml) x L of 24h urine
   µg coproporphyrins/24 hours=
   = [(F1 - F2)/(Fst - Fo)] x 5 x standard (µg/ml) x L of 24h urine
4. In some cases the coproporphyins washing is not total because of their chemical characteristics, and small quantities happened to be dosed together with uroporphyrins.
5. FAR kit to define U/C porphyrins shows a correlation coefficient of 0.996, in comparison to another kit available on the market.

REFERENCE

1. C. Sobel, C. Cano, and R.E. Thiers, Clin. Chem., Vol. 20, No. 11, 1397-1402. (1974)

IVD CE

Issue 02 - Jul 2023 MS

Manufactured by: **FAR** srl
Via Fermi, 12 - 37026 Pescantina - VERONA - ITALY
Phone +39 045 6700870
website: http://www.fardiag.com e-mail: fardiag@fardiag.com