PORPHYRINS T

Chromatographic – Spectrophotometric Determination
of Preformed or Total Porphyrins
(Preformed Porphyrins + Porphyrinogens)
in Urine

20 tests **REF KR09-20**

INTENDED USE

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

PRINCIPLE

Porphyrins are adsorbed on an anionic resin. Interfering substances are separated by washing and porphyrins are eluted and spectrophotometrical determined by calculating the concentration by Allen formula or by a fluorometer.

REAGENTS AND COLUMNS

Kit components:	REF KR09-20
***REAGENT 1** Diluted hydrochloric acid	**1 x 105 ml**
COLUMNS Chromatographic columns	**20**

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

STABILITY: stored at 20-25°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 REQUIRED BUT NOT SUPPLIED

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

SAMPLE

24 hour urine.
Store samples protected from light.

DETERMINATION OF PREFORMED PORPHYRINS

Perform the test immediately after urine collection, as porphyrinogens transformation into porphyrins may give misleading results. Porphyrinogens with 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
Optical path:	1 cm
Reading:	against Reagent 1
Temperature:	room temperature
Method:	spectrophotometric or fluorometric
Linearity:	up to 13 mg/L
Sensitivity:	colorimetrics 40 µg/L fluorometrics 10 µg/L
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 the column:

Urine	1.0 ml	discard the eluate
Distilled water	5.0 ml	discard the eluate

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

Reagent 1	2.5 ml	collect the eluate

Wait until the liquid has completely drained, then pipette once more:

Reagent 1	2.5 ml	collect the eluate

Collect the eluate together with the previous one.
Mix the eluates (5 ml) and read absorbencies at 380 nm, with maximum absorption between 400 and 407 nm and at 430 nm against the Reagent 1 (Allen correction).

CALCULATION

Calculate the difference between the absorption values (ΔA) measured according to the following formula:

ΔA = 2A (400 - 407 nm) - [A (380 nm) + A (430 nm)]

Porphyrins (µg/L) = ΔA x 3857

µg total porphyrins/L x L of 24h urine = µg total porphyrins / 24 hours

REFERENCE VALUES

Men: 50 - 200 µg/24 hours
Women: 35 - 180 µg/24 hours

NOTES

1. If sodium carbonate was added to the sample to bring it to alkaline pH, when adding Reagent 1 some CO_2 bubbles may form, slowing the liquid flow in the column. Eliminate the bubbles by lightly inclining the column.
2. To define porphyrins in 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 absorbencies 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 (F), of the working standard (Fst) and the Reagent 1 (Fo) and calculate as it follows:
   µg total porphyrins/24 hours =
   = [(F - Fo)/(Fst - Fo)] x 5.3 x standard (µg/ml) x L of 24h urine.
3. Urines preserved with sodium carbonate can not be used to define ALA or PBG, as they are not stable at a basic pH.
4. Any eventual pyrrolic substance in urine does not interfere: bilirubin is not eluted up to 10 mg/L concentration and hemine clorure up to 100 mg/L. Porphobilinogen is present in acid eluate, but there is no absorption in the spectral field between 360 and 450 nm.
5. FAR kit (y) shows a correlation coefficient of 0.998 to define porphyrins T, in comparison to another kit available on the market.

REFERENCE

1. M. Doss et U. Bode, Z. Klin. Chem. Klein. Biochem. 9, 415 (1971)

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