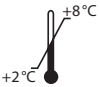
Manual

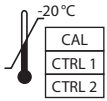
Porphyrins HPLC Kit

For the determination of porphyrins (free acids) in urine

Valid from 2023-01-12

REF **KCR2601**





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Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 70190-363
e.mail: info@immundiagnostik.com www.immundiagnostik.com

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1. INTENDED USE

This HPLC application is intended for the quantitative determination of porphyrins in urine. For research use only. Not for use in diagnostic procedures.

2. SUMMARY AND EXPLANATION OF THE TEST

The porphyrins are precursors of heme, which is a cofactor in hemoglobin, myo-globin and the cytochromes. They play an important role in the oxygen metabolism. Heme biosynthetic activity is located quantitatively in bone marrow and liver. The series of reactions leading to heme synthesis begins with the condensation of succinyl coenzyme A (CoA) and glycine and ends with the insertion of an iron atom into a molecule of protoporhyrin IX.

3. PRINCIPLE OF THE TEST

The first step in the determination of porphyrins includes an easy sample preparation. The pH value of the sample and the calibrator is adjusted below 2.5 by addition of 20 µl of hydrochloric acid. After centrifugation, 100 µl of the supernatant is injected into the HPLC system.

The separation via HPLC follows a gradient method, using a reversed phase column at 30 °C; one run lasts 25 minutes. The the chromatograms are recorded with a fluorescence detector. The quantification is performed with the delivered calibrator. The concentration is calculated via integration of the peak areas or peak heights.

Summary

This HPLC application provides an easy, fast and precise method for quantitative determination of porphyrins. The kit contains all reagents necessary for sample preparation and separation except the column.

As for many other parameters, the advantage of HPLC analytics is the simultaneous handling of many analytes in a single test. The HPLC complete system enables even laboratories without experience in high performance liquid chromatography to use this technique for clinical chemical routines quickly and precisely. Mostly, a one-point calibration is sufficient for calibrating the test system – unlike immunoassays with up to 6 calibrators per test. It is possible to automate the sample application and calculation of the results so that even higher number of samples can be handled nearly without control. With short test series, the one-point calibration is much more economic than 6-point calibration for immunoassays.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR0005.15	RECSOL	Reconstitution solution	1 x 15 ml
KCR2601	MOPHAA	Mobile phase A	1 000 ml
	MOPHAB	Mobile phase B	2 x 1 000 ml
	CAL	Calibrator; lyophilised (see specification data sheet for concentration)	4 x
	HCL	Hydrochloric acid	3 ml
	CTRL1	Control1; lyophilised	4 x
	CTRL2	Control2; lyophilised	4 x

For reorders of single components, use the catalogue number followed by the label as product number.

The HPLC column (KCR2601RP), can be ordered separately from Immundiagnostik. To extend the lifetime of your HPLC column, pre-columns (KCR2601VS) are highly recommended. These and also the pre-column holders (KCR2601VH) can also be ordered from Immundiagnostik.

In addition to the complete kits, all components can also be ordered separately. Please ask for our single component price list.

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- 1.5 ml reaction tubes (e. g. Eppendorf)
- Centrifuge
- Various pipettes
- Vortex
- HPLC gradient pump with fluorescence detector
- Reversed phase C_{18} column

6. STORAGE AND PREPARATION OF REAGENTS

- **The lyophilised calibrator** (**CAL**) is stable at **-20 °C** until the expiry date stated on the label. Before use, the **CAL** has to be reconstituted with **x µl (x = see the enclosed product specification for the volume needed) reconstitution solution** (**RECSOL**). The concentration of porphyrins slightly changes from lot to lot, the exact concentration is stated on the specification data sheet.
- **The lyophilised controls 1 and 2** (**CTRL1 and 2**) are stable at **-20 °C** until the expiry date stated on the label. Before use, they have to be reconstituted each with **x µl (x = see the enclosed product specification for the volume needed) reconstitution solution** (**RECSOL**). The concentration of porphyrins slightly changes from lot to lot, the exact concentration is stated on the specification data sheet.
- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at **2–8 °C**.

7. SPECIMEN COLLECTION AND PREPARATION

Urine is suited for this test system.

The porphyrins are light and temperature-sensitive. Therefore, protect samples from light and cool immediately after collection.

8. ASSAY PROCEDURE

Test procedure

Add **20 µl hydrochloric acid** (HCL) to the **calibrator** and **control 1 or 2** in the original flask and mix well. Pipet **250 µl** into a reaction tube.	Mix **250 µl** of a cool and dark stored **24 h urine** (without additives), with **20 µl hydrochloric acid** (HCL) (pH should be below 2.5) and mix well.
Centrifuge for **5 min** at **10 000 *g***.	
Inject 100 µl of the supernatant into the HPLC	

Chromatographic conditions

Column material:	Bischoff Prontosil 120-5-C18 ace EPS; 5 µm	
Column dimension:	125 mm × 4 mm	
Flow rate:	0.75 ml/min	
Fluorescence detection:	Excitation:	400 nm
	Emission:	620 nm
Temperature:	30 °C	
Injection volume:	100 µl	
Run time:	25 minutes	

Gradient:

Time	% B	% A
0 min	38	62
1.5 min	38	62
9.5 min	80	20
11.5 min	80	20
11.6 min	95	5
15 min	95	5
15.1 min	38	62
24 min	38	62

9. TREATMENT OF THE COLUMN

It is recommended to use a guard column to extend column life.

After analysis, the column should be flushed with 30 ml ultra pure water (1 ml/min) and stored in 50% methanol in water (~ 30 ml, flow 0.7 ml/min). Before use, the system should be equilibrated with ~ 30 ml mobile phase (MOPHA).

10. RESULTS

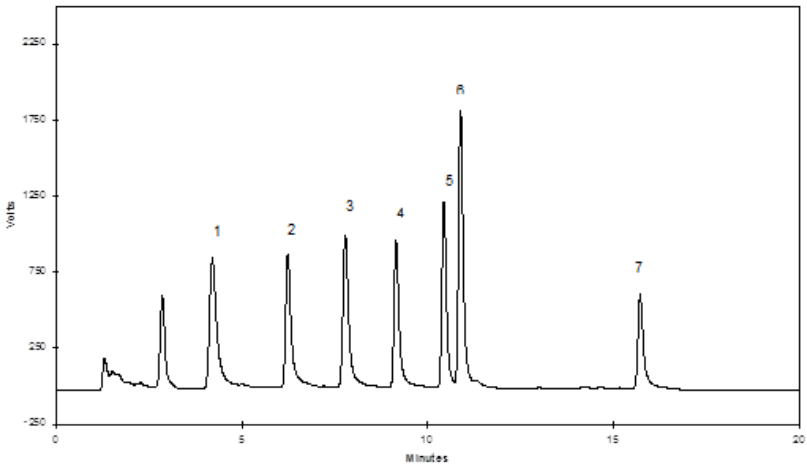
Calculation

$$\text{Sample concentration} = \frac{\text{Peak height sample} \times \text{calibrator concentration*}}{\text{Peak height calibrator}}$$

* see specification data sheet

Tip: Alternatively, the peak area instead of the peak height can be used for quantification.

Typical chromatogram



Column: Prontosil 120-5-C18 ace-EPS

Order of peaks (from the left to the right)

1) 8-carboxyl Porphyrin (Uro-)
2) 7-carboxyl Porphyrin (Hepta-)
3) 6-carboxyl Porphyrin (Hexa-)
4) 5-carboxyl Porphyrin (Penta)
5) 4-carboxyl Porphyrin (Copro) I
6) 4-carboxyl Porphyrin (Copro) III
7) Mesoporphyrin IX

11. LIMITATIONS

Do not use blood samples for analysis.

12. QUALITY CONTROL

Reference ranges

We recommend each laboratory to establish its own reference range. The above mentioned values are only for orientation and may deviate from other published data.

Controls

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

13. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay CV:

Uroporphyrin	5.6 % (35.8 µg/l)	[n = 10]
Heptaporphyrin	2.5 % (25.3 µg/l)	[n = 10]
Hexaporphyrin	2.6 % (27.0 µg/l)	[n = 10]
Pentaporphyrin	2.6 % (31.7 µg/l)	[n = 10]
Coproporphyrin I	2.5 % (24.9 µg/l)	[n = 10]
Coproporphyrin III	2.6 % (57.1 µg/l)	[n = 10]

Inter-Assay CV:

Uroporphyrin	6.6 % (52.5 µg/l)	[n = 5]
Heptaporphyrin	6.0 % (30.1 µg/l)	[n = 5]
Hexaporphyrin	5.0 % (30.8 µg/l)	[n = 5]
Pentaporphyrin	5.1 % (35.9 µg/l)	[n = 5]
Coproporphyrin I	7.5 % (24.0 µg/l)	[n = 5]
Coproporphyrin III	4.7 % (65.9 µg/l)	[n = 5]

14. DISPOSAL

The mobile phase (MOPHA) must be disposed as non-halogenated solvent. The hydrochloric acid (HCL) solution can be neutralized with NaOH and if the pH value is neutral it can be disposed of as salt solution.

Important: Reaction will produce heat, be careful!

Please refer to the appropriate national guidelines.

15. TROUBLESHOOTING

Problem	Possible cause	Solution
No signal	No or defect connection to evaluation system	Check signal cord and connection
	Detector lamp is too old	Change lamp
No peaks	Injector is congested	Check injector
Double peaks	Dead volume in fittings and / or column	Renew fittings and / or column
Contaminating peaks	Injector dirty	Clean injector
	Contamination at the head of the column	Change direction of the column and rinse for 30 min at low flow rate (0.2 ml/min) with mobile phase
	Air in the system	Degas pump
	Auto sampler vials contaminated	Use new vials or clean them with methanol
Broad peaks, tailing	Precolumn / column exhausted	Use new precolumn / column
Variable retention times	Drift in temperature	Use a column oven
	Pump conveys inaccurately	Check pump, degas the system
	System is not in steady state yet	Rinse system mobile phase for 15 min

Problem	Possible cause	Solution
Baseline is drifting	Detector lamp did not reach working temperature yet	Wait
	Detector lamp is too old	Change lamp
	System is not in steady state yet	Rinse system mobile phase for 15 min
	Pump conveys inaccurately	Check pump, degas the system
Baseline is not smooth	Pump conveys inaccurately	Check pump, degas the system
	Detector flow cell is dirty	Clean flow cell

16. PRECAUTIONS

- All reagents in the kit package are for research use only.
- The hydrochloric acid (HCL) solution contains acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.
- As a precaution, it is recommended that the human material used is always considered potentially infectious.
- The test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- Reagents should not be used beyond the expiration date shown on kit label.

17. GENERAL NOTES ON THE TEST

- The assay should always be performed according to the enclosed manual.
- Plugs and caps of different reagents should not be swapped.
- Quality control guidelines should be observed.
- Do not interchange different lot numbers of any kit component within the same assay.
- The guidelines for laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which has not been consulted with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be sent to Immundiagnostik AG along with a written complaint.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.

18. REFERENCES

1. Armbruster et al. (1983). Auftrennung und Quantifizierung der Porphyrine mit Hilfe der Hochleistungs-Flüssigkeits-Chromatographie. *Ärztl. Lab*. **29**; 379-384.
2. Kazuyuki O. et al. (1988). Reevaluation of urinary excretion of coproporphyrins in lead-exposed workers. *Int Arch Occup Environ Health* **60**; 107-110.
3. Thomas L. (Hrsg). *Labor und Diagnose* **5. Auflage** S. 458-474.

Used symbols:


Temperature limitation

Catalogue number

For research use only

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Contains plasma derivatives or human blood


Consult instructions for use

Consult specification data sheet


Do not re-use

Unique Device Identification

Contains material of animal origin


Medicinal substance

Contains material of human origin

Immundiagnostik AG

Stubenwald-Allee 8a
64625 Bensheim, Germany

Tel.: +49 6251 70190-0
Fax: +49 6251 70190-363

info@immundiagnostik.com
www.immundiagnostik.com