



EAGLE
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Porphyrins HPLC Assay

Catalog Number: PPR34-H100

100 Tests

For Research Use Only. Not for use in diagnostic procedures.

v. 1.0

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1. Intended purpose

The Eagle Biosciences Porphyrins HPLC Assay Kit is intended for the quantitative determination of porphyrins in urine. This Porphyrins HPLC Assay Kit is for research use only and is not for use in diagnostic procedures.

2. Introduction

Hemoglobin, myoglobin and the cytochromes contain the heme structure as active site. The porphyrins are precursors of the heme molecules. They play an important role in the oxygen metabolism. The heme synthesis is located mainly in the erythrocyte cells. Glycine and succinyl-CoA are condensed to δ -aminolävulinic acid. Two molecules of δ -aminolävulinic are then condensed to porphobilinogen. The porphyrin ring is built up by four porphobilinogen molecules.

An increase of porphyrins caused by a defect in the synthesis is described as porphyria. The different porphyries are determined by the characteristic pattern of porphyrins in urine. In autosomal dominant acute porphyrin disorders uro-, copro-, penta- und tricarboxyporphyrin are increased. In chronic porphyries including porphyria cutanea tarda the concentration of uro- and heptaporphyrin are increased. In chronic lead intoxication the coproporphyrins are slightly increased, whereas an acute lead intoxication shows extremely high excretion of porphyrins in urine.

The Eagle Biosciences Porphyrins HPLC Assay Kit makes it possible to determine them in an easy, fast and precise way. The Porphyrins HPLC Assay Kit includes all reagents in ready to use form for preparation and separation of the samples with exception of the columns (IC2601rp) and the controls (IC2601ko). Both can be supplied by Eagle Biosciences. Beside the complete test kit it is possible to order all components separately. Please request our single component price list.

3. Warnings and precautions

- All reagents of this Porphyrins HPLC Assay Kit are strictly intended for research use only.
- Test kit and column are concerted. Using alternative columns might cause in insufficient separation, resulting in false high results. The given test characteristics might not be fulfilled.
- Do not interchange the Eagle Biosciences Porphyrins HPLC Assay Kit components from different lots.



- The stabilization solution contains acid and has to be handled carefully. It is corrosive and causes burns. It should be handled with gloves, eye protection and appropriate protective clothing in a hood. Any spill should be wiped out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation. In case of an accident or indisposition immediately contact a physician.
- The mobile phase B contains methanol and has to be handled carefully. Methanol is highly flammable and toxic if inhaled or contact the skin. It should be handled with gloves, eye protection, and appropriate protective clothing in a hood. Any spill should be wiped out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation. In case of an accident or indisposition immediately contact a physician.
- The mobile phase A contains a preservative against bacterial growth. At the product concentration, though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices.
- Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
- Porphyrins HPLC Assay Kit reagents should not be used beyond the expiration date shown on kit label.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled urine. During handling of all kit reagents, controls and urine samples observe the existing legal regulations.

4. Materials Provided

Article no.	Component	Designation	Amount
IC2601la	ELUA	Mobile phase A	1000 ml
IC2601lb	ELUB	Mobile phase B	2000 ml
IC2601ka	CAL	Calibrator, (lyoph. 1 ml)	10 vials
IC2601re	RECON	Reconstitution solution	10 ml
IC2601hc	HCL	Stabilization solution (contains HCl – caution corrosive)	10 ml



5. Additional special equipment

- 1.5 ml reaction tubes (Eppendorf)
- Centrifuge
- Various pipettes
- HPLC gradient system with Fluorescence-detector
- HPLC column Porphyrins (IC2601rp)
- Vortex mixer

6. Reagent preparation

- Reconstitute the **calibrator (CAL)** in **1 ml** reconstitution solution (RECON), divide the calibrator in several portions and store them at -20 °C. Avoid repeated freeze-thaw circles. The concentration of porphyrins might have minor changes from lot to lot. The exact concentration is given on the Porphyrins HPLC Assay Kit product specification.
- All other Porphyrins HPLC Assay Kit reagents are stable at 2-8 °C, up to the date of expiry stated on the label.

7. Specimen

- Urine could be used in this test system.
- The porphyrins are light- and temperature sensitive; therefore samples have to be protected from light and cooled immediately.
- The samples are stable in the dark at 2-8°C for 1 week. For longer storage samples should be frozen at -20 °C.

8. Procedure

Principle of the method

For the determination of the porphyrins a very easy sample preparation is performed first. The pH value of the sample, calibrator and control is adjusted below 2.5 by addition of some drops (approx. 20 µl) of a stabilization solution. After centrifugation the supernatant is injected into the HPLC system. The separation via HPLC follows an gradient method at 30 °C, using a "reversed phase" column; one run lasts 25 minutes. The quantification is performed with the delivered calibrator; the concentration is calculated via integration of the peak areas or peak heights.



Sample preparation

1. Pipette into 1.5 ml reaction tubes:
1000 µl sample, CAL or CTRL
+
20 µl HCL
2. Mix well (pH value should be less than 2.5). Centrifuge at 10.000g for 5 minutes.
3. Inject **100 µl** of the supernatant for chromatography into the HPLC-system

Chromatographic settings

Column material:	Prontosil ace EPS, 5 µm
Column dimension:	125 mm x 4 mm
Flow rate:	0.75 ml/min
Fluorescence detection:	Excitation 400 nm Emission 620 nm
Injection volume:	100 µl
Running time:	25 min
Temperature:	30 °C
Gradient:	
	0 min (38% B / 62 % A)
	1.5 min (38% B / 62 % A)
	9.5 min (80% B / 20 % A)
	11.5 min (80% B / 20 % A)
	11.6 min (95% B / 5 % A)
	15 min (95% B / 5 % A)
	15.1 min (38% B / 62 % A)
	24 min (38% B / 62 % A)

We recommend using a precolumn to enlarge the lifetime of the analytical column.

Treatment of the HPLC column

After the analysis the column should be flushed with 15 ml 50% methanol / deionized water (approx. 15 ml, flow 0.7 ml/min) and closed tightly. Before use, the system should be equilibrated with approx. 30 ml eluent (38% B / 62% A).

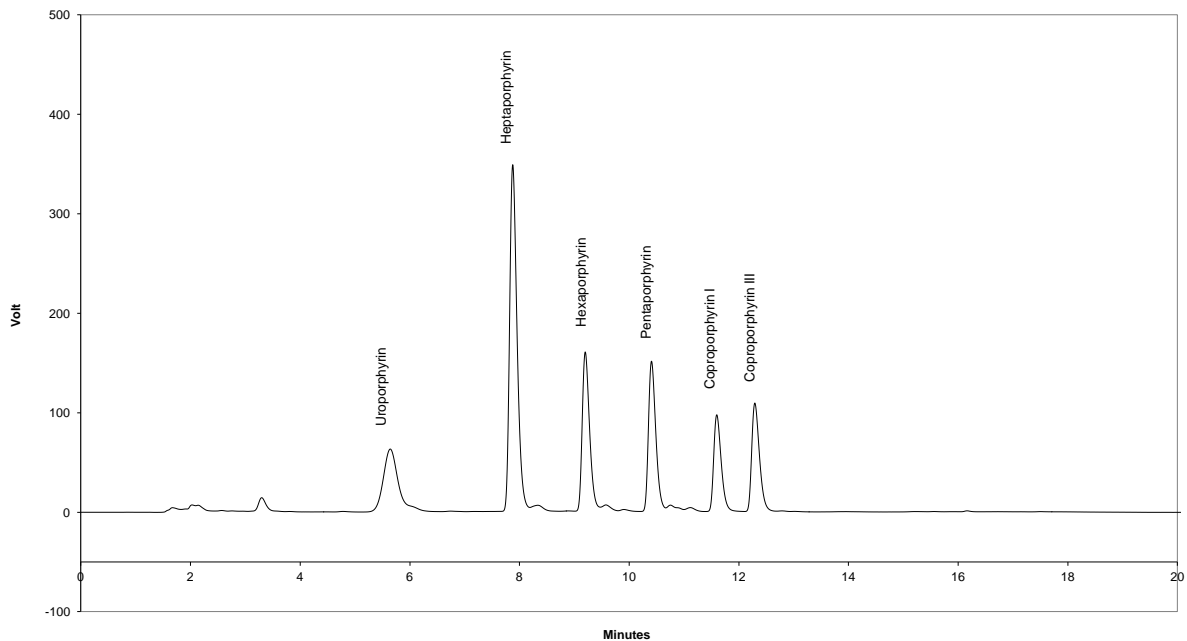


11. Calculation of analytical results

Calculation

$$\text{Conc. sample } (\mu\text{g/l}) = \frac{\text{peak area patient} * \text{conc. calibrator } (\mu\text{g/l})}{\text{peak area calibrator}}$$

Typical chromatogram



10. Internal quality control

Reference intervals

The normal range is related to a 24 h urine.

	Mean ($\mu\text{g}/24 \text{ h}$)	Range ($\mu\text{g}/24 \text{ h}$)
8-carboxyl Porphyrin (Uro-)	7.0	0-33
7-carboxyl Porphyrin (Hepta-)	< detection limit	0-10
6-carboxyl Porphyrin (Hexa-)	< detection limit	0-7
5-carboxyl Porphyrin (Penta)	< detection limit	0-5
4-carboxyl Porphyrin (Copro)	44.8	0-120
Coprop. I	17 – 31 %	
Coprop. III	69 – 83 %	



We recommend that each laboratory should develop their own normal range. The values mentioned above are only for orientation and can deviate from other published data.

(Armbruster et al. (1983). Auftrennung und Quantifizierung der Porphyrine mit Hilfe der Hochleistungs-Flüssigkeits-Chromatographie. *Ärztl. Lab.* 29; 379-384. Thomas L. (Hrsg). *Labor und Diagnose* 5. Auflage S. 458-474)

14. Validation data

Precision and reproducibility

Intra-Assay CV:

Uroporphyrin	3.6 % (34.3 µg/l)	1.8 % (66.9 µg/l)	[n = 6]
Heptaporphyrin	0.8 % (42.7 µg/l)	2.0 % (96.3 µg/l)	[n = 6]
Hexaporphyrin	1.2 % (21.4 µg/l)	1.7 % (48.7 µg/l)	[n = 6]
Pentaporphyrin	1.2 % (20.9 µg/l)	1.9 % (55.5 µg/l)	[n = 6]
Coproporphyrin I	1.6 % (23.7 µg/l)	1.8 % (38.3 µg/l)	[n = 6]
Coproporphyrin III	1.5 % (21.3 µg/l)	1.9 % (81.1 µg/l)	[n = 6]

Inter-Assay CV:

Uroporphyrin	6.0 % (35.4 µg/l)	3.9 % (65.0 µg/l)	[n = 6]
Heptaporphyrin	3.3 % (42.9 µg/l)	2.5 % (96.2 µg/l)	[n = 6]
Hexaporphyrin	1.2 % (21.6 µg/l)	1.8 % (48.7 µg/l)	[n = 6]
Pentaporphyrin	1.7 % (20.8 µg/l)	2.2 % (55.0 µg/l)	[n = 6]
Coproporphyrin I	1.6 % (23.6 µg/l)	2.4 % (37.9 µg/l)	[n = 6]
Coproporphyrin III	1.8 % (21.0 µg/l)	2.2 % (80.3 µg/l)	[n = 6]

Linearity

up to 1 mg/l

Detection limit

0.4 µg/l

Recovery

99.0 – 102.1 %

12. Limitations of the method

Blood samples should not be analyzed.



13. Disposal

The mobile phase B (ELUB) must be disposed as non-halogenated solvent. The stabilization solution (STAB) could be neutralized with NaOH and if the pH value is neutral it can be disposed as salt solution. (**Important:** Reaction will produce heat, be careful). Please refer to the appropriate national guidelines.

14. TROUBLESHOOTING

Problem	Possible reason	Solution
No signal	No or defect connection to evaluation system	Check signal cord and connections
	Lamp of detector is altered	Renew lamp
No peaks	Injector is congested	Check injector
Double peaks	Dead volume in fittings and/or at the head of 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 the mobile phase and pump head
	Autosampler vials contaminated	Use new vials or clean them with methanol
Broad peaks, tailing	Precolumn / column exhausted	Renew precolumn / column
Variable retention times	Drift in temperature	Use a column oven
	Pump delivers imprecise	Check pump, degas the system
	System is not in steady state yet	Rinse system mobile phase for 15 min
Baseline is drifting	Detector lamp did not reach working temperature yet	Wait
	Detector lamp is too old	Renew lamp
	System is not in steady state yet	Rinse system mobile phase for 15 min
	Pump delivers imprecise	Check pump, degas the system
Baseline is not smooth	Pump delivers imprecise	Check pump, degas the system
	Detector flow cell is dirty	Clean flow cell
	Detector lamp is too old	Renew lamp



15. LITERATURE REFERENCES

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

For further information about this kit, its application or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.