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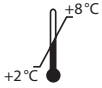
Manual

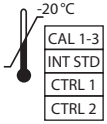
Zinc-Protoporphyrin/ Protoporphyrin HPLC Kit

For the determination of zinc-protoporphyrin/ protoporphyrin in EDTA whole blood

Valid from 2022-09-06

REF **KC2700**





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1. INTENDED USE

This HPLC application is intended for the quantitative determination of zinc-protoporphyrin and protoporphyrin in EDTA whole blood. For *in vitro* diagnostic use only.

2. SUMMARY AND EXPLANATION OF THE TEST

Zinc protoporphyrin (ZPP) is a metabolite formed in erythrocytes during hemoglobin synthesis. By iron deficiency during erythropoiesis, instead of incorporating a ferrous ion to form the hem precursor, zinc becomes an alternative metal ion in the protoporphyrin complex. As a result, zinc-protoporphyrin is produced instead of hemoglobin. As a consequence, in cases of iron deficiency anemia, the zinc protoporphyrin concentration in the erythrocytes is elevated.

Indications

- Lead poisoning
- Iron deficiency
- Sickle cell anemia
- Sideroblastic anemia
- Anemia of chronic disease
- Vanadium exposure

3. PRINCIPLE OF THE TEST

To determine the zinc protoporphyrin or the protoporphyrin, a cell disruption is the first step. In a second step, precipitation is carried out to separate higher-molecular substances. After their removal by centrifugation, the supernatant is injected into the HPLC system.

The separation via HPLC follows an isocratic method at 30 °C using a reversed phase column. One run lasts 10 minutes. The chromatograms are recorded by a fluorescence detector. The quantification is performed with the delivered calibrators and the calculation of the results is carried out via the external standard method, using the integration of the peak area and a calibration line.

In parallel measurements of the hemoglobin concentration are required, because the zinc-protoporphyrin concentration is related to the hemoglobin concentration.

Summary

This HPLC technique provides an easy, fast and precise method for quantitative determination of zinc-protoporphyrin and protoporphyrin. Except the column, the kit contains all reagents necessary for sample preparation and separation in ready-to-use form.

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. 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.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 0005.15	RECSOL	Reconstitution solution	15 ml
KC2700	CAL1	Calibrator1; lyophilised (see specification data sheet for concentration)	4 x
	CAL2	Calibrator2; lyophilised (see specification data sheet for concentration)	4 x
	CAL3	Calibrator3; lyophilised (see specification data sheet for concentration)	4 x
	CTRL1	Control1; lyophilised	4 x
	CTRL2	Control2; lyophilised	4 x
	INTSTD	Internal Standard; lyophilised	4 x
	MOPHA	Mobile phase (important: do not recirculate)	2 x 1 000 ml
	PREC	Precipitation reagent; lyophilised	1 x
	SOLA	Lysis reagent	20 ml

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

The HPLC column (KC2700RP), can be ordered separately from Immundiagnostik. To extend the lifetime of your HPLC column, pre-columns (KC2700VS) are highly recommended. These and also the pre-column holders (KC2700VH) 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

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

6. STORAGE AND PREPARATION OF REAGENTS

- **The lyophilised calibrators** (**CAL 1–3**) is stable at **-20 °C** until the expiry date stated on the label. Before use, the CAL 1–3 has to be reconstituted with **500 µl reconstitution solution** (RECSOL). The concentration of zinc-protoporphyrin slightly changes from lot to lot, the exact concentration is stated on the specification data sheet. **Calibrator** (reconstituted CAL 1–3) **is not stable and cannot be stored.**
- **The lyophilised controls 1 and 2** (**CTRL 1 and CTRL 2**) are stable at **-20 °C** until the expiry date stated on the label. Before use, they have to be reconstituted with each **250 µl reconstitution solution** (RECSOL). The concentration of zinc-protoporphyrin slightly changes from lot to lot, the exact concentration is stated on the specification data sheet. **Controls** (reconstituted CTRL 1 and 2) **are not stable and cannot be stored.**
- **The lyophilised internal standard** (**INT STD**) is stable at **-20 °C** until the expiry date stated on the label. Before use, the INT STD has to be reconstituted with **2 ml precipitation reagent** (PREC). **Internal standard** (reconstituted INT STD) **is not stable and cannot be stored.**
- 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

EDTA whole blood is suited for this test. Before analysis, the erythrocytes are lysed by freezing and thawing in order to release zinc-protoporphyrin and protoporphyrin.

Zinc-protoporphyrin as well as protoporphyrin are light sensitive. Samples should be transported lightproof.

8. ASSAY PROCEDURE

Test procedure

1.	homogenise samples well, e.g. on a shaker for ~ 3 min.
2.	fill 100 µl **sample**, **CAL 1–3** or **CTRL 1–2** into 2 ml reaction tubes
3.	add 150 µl **SOL A** each
4.	add 10 µl **INT STD** each and mix well (vortex for at least 30 s)
5.	incubate for 5 min at room temperature
6.	quickly add 1 000 µl **PREC** each, mix well (vortex for at least 30 s)
7.	centrifuge for 10 min at 4 °C and 10 000 rcf
8.	transfer ~ 190 µl supernatant into HPLC vials with inserts

Chromatographic conditions

Column material:	Bischoff Prontosil 120-5-C18 ace EPS; 5 µm	
Column dimension:	125 mm × 4 mm	
Flow rate:	2.0 ml/min	
Fluorescence detection::	Excitation:	417 nm
	Emission:	635 nm
Temperature:	30 °C	
Injection volume:	50 µl	
Running time:	4.2 min	

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 0.5 ml/min). Before use, the system should be equilibrated with ~ 60 ml mobile phase (MOPHA).

10. RESULTS

Calculation

The obtained peak areas are normalised to the area of the internal standard of the respective sample.

$$y = \frac{y_a}{y_i}$$

With the normalised signal of the calibrators (**CAL 1–3**) and the concentration values (see specification data sheet) a calibration line has to be created. This calibration line has the following calibration function:

$$\frac{y_a}{y_i} = b_0 + b_1 \times c_A$$

The calculation of the concentration of the analyte in the samples and the **CTRL 1** and **CTRL 2** is then done with the following formula:

$$c_A = \frac{(y_A/y_i) - b_0}{b_1}$$

b_0 = y-axis intercept of the calibration line

b_1 = slope of the calibration line

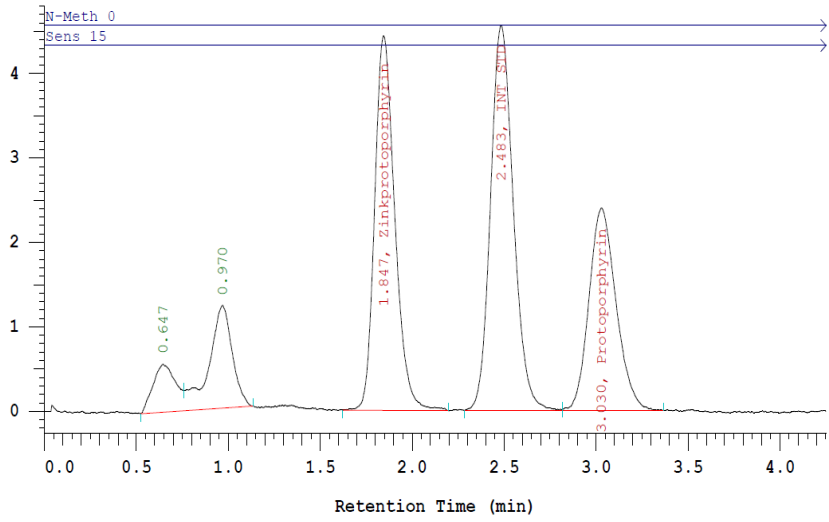
y = signal

A = analyte (zinc protoporphyrin or protoporphyrin)

i = internal standard

c = concentration

Typical chromatogram



11. QUALITY CONTROL

Reference range

	Zink-Protoporphyrin [µmol/mol Häm]
adequate supply of iron	0–80
inadequate supply of iron possible, confirmation by further parameter strongly recommended	> 80 < 160
inadequate supply of iron	> 160

We recommend each laboratory to establish its own reference range. The values mentioned above 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 patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

12. PERFORMANCE CHARACTERISTICS

Analytical sensitivity

LoD Zink-Protoporphyrin:	32.4 nmol/l
LoD Protoporphyrin:	6.6 nmol/l
LoQ Zink-Protoporphyrin:	108 nmol/l
LoQ Protoporphyrin:	22.1 nmol/l

Analytical specificity

There were no known interferences to other blood components found.

13. DISPOSAL

The mobile phase (**MOPHA**) and precipitation reagent (**PREC**) must be disposed as non-halogenated solvents.

Please refer to the appropriate national guidelines.

14. TROUBLESHOOTING

Problem	Possible cause	Solution
No signal	No or defect connection to evaluation system	Check signal cord and connection
	Detector lamp is altered	Change lamp

Problem	Possible cause	Solution
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 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 for operating temperature
	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

15. PRECAUTIONS

- 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.
- The test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- As a precaution, it is recommended that the human material used is always considered potentially infectious.

16. GENERAL NOTES ON THE TEST

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated 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.
- The assay should always be performed according the enclosed manual.
- Control samples should be analysed with each run.
- Plugs and caps of different reagents should not be swapped.
- Do not interchange different lot numbers of any kit component within the same assay.
- The guidelines for medical laboratories should be followed.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.
- Reagents should not be used beyond the expiration date stated on kit label.

17. REFERENCES

1. Hastka, J., Heimpel, H., & Metzgeroth, G. (2011). Eisenmangel und Eisenmangelanämie. DGHO - Deutsche Gesellschaft für Hämatologie und klinische Onkologie.

Used symbols: