PROTOPORPHYRIN IX

Quantitative Fluorometric Determination
of Free Erythrocytic Porphyrins
in Blood

20 tests **REF KR10-20**

INTENDED USE

Kit for quantitative *in vitro* determination of Free Erythrocytic Porphyrins in the blood.

PRINCIPLE

Free erythrocytic porphyrins, in contact with an adsorbent to ease interfering substances retention, are quantitatively extracted with an ethyl acetate-acetic acid mixture. Once transferred in hydrochloric acid, their concentration is fluorometrically defined.

REAGENTS AND MATERIALS

Kit components:	REF KR10-20
***REAGENT 1** Solvent	**1 x 40 ml**
***REAGENT 2 standard** Concentrated protoporphyrin IX with concentration of 500 µg/100 ml	**1 x 5 ml**
***REAGENT 3** Hydrochloric acid 1.5 mol/L	**1 x 84 ml**
***REAGENT 4** Hydrochloric acid-ethyl acetate	**1 x 40 ml**
TEST-TUBES Tubes containing adsorbent	**20**

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

STABILITY: stored at 2-8°C, sealed reagents and test-tubes are stable up to the expiration date on the label.

EQUIPMENT REQUIRED BUT NOT SUPPLIED

Centrifuge, spectrophotometer or filter photometer (408 nm), spectrofluorometer/filter fluorometer (excitation 405 nm, emission 600 nm).

PREPARATION OF THE STANDARDS

STANDARD 100 µg/dl

To prepare the protoporphyrin IX standard (100 µg/100 ml) , dilute 1 volume of Reagent 2 with 4 volumes of Reagent 3 and shake.
The following volumes are recommended:

Reagent 2 Standard	0.5 ml
Reagent 3	2.0 ml

The obtained volume of 2.5 ml is enough to prepare 25 doses of Standard 5.
Read the A Std (100) absorbance at 405 nm against the Reagent 3.
Calculate the K correction coefficient : K = A Std(100) / 0.489.
STABILITY: stable for some weeks if stored at 2-8°C and protected from light.

STANDARD 5 µg/dl

To prepare protoporphyrin IX standard with concentration of 5 µg/100 ml, dilute 1 volume of Standard 100 with 19 volumes of Reagent 4.
The following volumes are recommended:

Standard 100	0.1 ml
Reagent 4	1.9 ml

The obtained volume of 2.0 ml of Standard 5 is to be used in the test.
WARNING: prepare this standard immediately before performing the test.

SAMPLE

Whole blood.
Use capillary blood or venous blood with heparin or EDTA as anticoagulant.

STABILITY: 72 hours for capillary blood at room temperature, 1 week at 2-8°C for heparinized or EDTA blood.

MANUAL ASSAY PROCEDURE

Wavelength:	excitation 405 nm / emission 600 nm
Reading:	against Reagent 3
Temperature:	room temperature
Method:	fluorometric
Linearity:	up to 2 mg/100 ml
Sensitivity:	3 µg/100 ml
C.V. (intra-assay):	2%
C.V. (inter-assay):	4%

Pipette into a disposable test-tube supplied with the kit:

Blood	20 µl
Reagent 1	2.00 ml

Shake the tube on Vortex for about 15 seconds and centrifuge for 2 minutes at 2000 rpm. Transfer the supernatant into a centrifuge tube and add:

Reagent 3	2.00 ml

Shake on Vortex for about 15 seconds and centrifuge for 2 minutes at 2000 rpm. Discharge the upper layer (organic) and collect the lower (watery) into a fluorometer cuvette. Read the sample fluorescence within 1 hour and compare it with the STANDARD 5's.
With analogical fluorometers, adjust the sensitivity in order to have the STANDARD 5 to read 67.5 x K.
With digital fluorometers, adjust the sensitivity in order to have the STANDARD 5 to read 675 x K.

CALCULATION

The samples reading, according to the above mentioned instruction, directly gives the concentration in µg of protoporphyrin IX/100 ml.

REFERENCE VALUES

up to 60 µg/100 ml of blood
up to 5.3 µg/g of Hb

NOTES

1. In cases of anaemia (with an evident red cell decrease, compared to normal conditions), it is recommended to define the hemoglobin concentration (Hb g/100 ml) and then divide the porphyrin value by the hemoglobin concentration.
2. Clinical significance:
   60-190 µg protoporphyrin IX/100 ml blood, 5.3-17 µg/g Hb:
   lead intoxication, iron-deficiency or sideropenic anaemia;
   > 190 µg protoporphyrin IX/100 ml blood, > 17 µg/g Hb:
   severe lead poisoning or rarely genetic disorders.
3. As protoporphirin IX is sensitive to light, define the standard concentration each time, considering the elective absorbance of the product. Using an instrument set for wavelength, a 100 µg/100 ml solution of protoporphirin IX, in HCl 1.5 M, gives an absorbance of A = 0.489.
   Therefore, K value can define the exact concentration of the prepared standard (=K x 100 µg/100 ml), which will be diluted 1:20 for the fluorimetric reference.
4. Due to division phenomena, after the extraction of Reagent 3, a final volume of the lower layer = 2.7 ml is obtained. Free porphirins extracted, contained in the 20 µl sample, are present in them, with a dilution ratio of 1:135. Considering moreover that STANDARD 5 concentration is =
   K x 5 µg/100 ml (see note 3), the concentration of Cc sample, related to the read fluorescences Fs and Fc (respectively for the standard and for the sample), is expressed in the formula:
   Cc = [(Fc x 135) x (K x 5)]/Fs, that is: Cc = Fc x [(K x 675)]/Fs,
   from which it results that for Fs=K x 675, the ratio is (K x 675)/Fs =1,
   and then Cc = Fc.
   Therefore, setting the instrument sensitivity to have STANDARD 5 fluorescence (Fs) to read K x 675, the sample readings in fluorescence directly reflects the respective concentration values in µg/100 ml. Fluorometers with analogical reading require setting at K x 67.5.

REFERENCE

1. S. Piomelli, "Clin. Chem." 23 (2), 264-269 (1977)

IVD CE

Issue 01 - Nov 2021

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