GLYCOSYLATED HEMOGLOBIN A_{1c} – PRONTO

Chromatographic – Spectrophotometric
Determination of Hemoglobin A1c in Blood
Ion Exchange - Independent Temperature method

40 tests **REF 3609**

INTENDED USE

Kit for quantitative *in vitro* determination of Hemoglobin A_1 in blood.

PRINCIPLE

Mixing a part of whole blood with a hemolysis reagent, red blood cells lysis is obtained, with hemoglobin release and labile fraction removal.
Hemoglobin A_{1c} fraction is then separated from HbA1A+B by cationic exchange chromatography.
HbA_{1c} percentage in the sample is obtained by the comparing ratio of the eluated absorbance (at 415 nm) with total hemoglobin absorbance.
The procedure doesn't require any calibration and can be performed with a temperature range of 20-28°C.

REAGENTS AND COLUMNS

Kit components:	REF 3609
REAGENT 1 Pothassium biphtalate	**1 x 10 ml**
***REAGENT 2** Buffer 30 mmol/L, LiCl, pH 6	**1 x 160 ml**
***REAGENT 3** Buffer 30 mmol/L, LiCl, pH 6	**1 x 160 ml**
COLUMNS Chromatographic columns	**40**

NOTE: use only reagents and columns of the same batch.

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

STABILITY: stored at 15-30°C, reagents and columns are stable up to the expiration date on the label. Store columns in the dark.

SAMPLE

Whole blood collected with EDTA or oxalate fluoride. Do not use heparin as anticoagulant.
STABILITY: 7 days at 2-8°C.

MANUAL ASSAY PROCEDURE

Wavelength:	415 nm
Optical path:	1 cm
Reading:	against distilled water
Temperature:	20-28°C
Method:	spectrophotometric
C.V. (intra-assay):	< 1.5%
C.V. (inter-assay)	< 4.0%

NOTE: Perform the assay with a temperature ranged between 20 and 28°C. Reagents and columns must have the same temperature before use.

PREPARATION OF HEMOLYSED SOLUTION

Pipette in a test tube:

Blood	0.050 ml
Reagent 1	0.200 ml

Mix vigorously and incubate at room temperature (20-28°C) for 10-15 minutes.
NOTE: use the hemolysed solution within 2 hours from its preparation.

PREPARATION OF THE COLUMN

Remove the cap and re-suspend the resin completely, pipetting the columns contents with a 5 ml pipette. Snap the column bottom tip off, place the columns in vertical position in a proper container and let the liquid completely drain, then discard it.

WARNING: during the whole chromatographic separation procedure, do not leave the resin without buffer for more than 5 minutes.

CHROMATOGRAPHIC SEPARATION

WARNING: during this step it is important to gently pipette the hemolysed solution and buffers into the column, to avoid the resin bed rising; therefore it is advisable to pipette the liquid onto the inner sides of the column, at 3 cm from the resin.
Pipette into the column:

Hemolysed solution	0.050 ml	Wait for 3 minutes. Discard any eventual eluate

Pipette into the column:

Reagent 2	4.0 ml	discard the eluate

Place the column over a test tube (16 x 160 mm) and pipette:

Reagent 3	4.0 ml	collect the eluate (HbA1c fraction)

Mix the obtained eluate and read the HbA1c fraction absorbance at 415 nm against distilled water (A Hb A1c).

PREPARATION OF TOTAL HEMOGLOBIN

Pipette in a test tube (16 x 160 mm):

Hemolysed solution	0.050 ml
Distilled water	12.0 ml

Mix and read the total hemoglobin (A Hb TOTAL) absorbance at 415 nm against distilled water.

CALCULATION

(A Hb A1c) / (3 x A Hb TOTAL) x 100 = % HbA1c

REFERENCE VALUES

Normal range: 4.2 - 6.2 %
These values are only indicative; each laboratory should define its own reference values.

NOTES

1. Interferences: wrong values may result from samples with abnormally high quantities of other hemoglobins due to their simultaneous elution with HbA1c (HbF) or to their differences in glication compared with HbA's (HbS).
2. The comparison between FAR kit (Y) with another kit (X) available on the market to define A1C glycosilated hemoglobin, gave the following correlation line:
   Y=1.45X-3 R = 0.957
3. Disposal of all waste material should be in accordance with the law.

REFERENCE

1. Mayer et Freedman (Clin.Chim.Acta, 1983; 127: 147-184)

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