GLYCOSYLATED HEMOGLOBIN A_{1c} – PRONTO

Chromatographic – spectrophotometric
determination of Hemoglobin A1c in blood
Ion exchange - Independent temperature method

20 tests REF **KR05-20**

INTENDED USE

Kit for quantitative *in vitro* determination of Hemoglobin A_{1c} in blood.

PRINCIPLE

By mixing a quote of whole blood with a hemolyzing reagent to obtain the lysis of the red blood cells is obtained, with also the release of hemoglobin and the elimination of the labile fraction.
Then hemoglobin A_{1c} fraction is separated from HbA1A+B by cationic exchange chromatography.
The ratio between the eluated absorbance (at 415 nm) and the total hemoglobin absorbance provide the percentage of HbA_{1c} in the sample.
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 KR05-20
REAGENT 1 Pothassium biphtalate	**1 x 10 ml**
REAGENT 2 Buffer 42 mmol/L, LiCl, pH 5.96	**1 x 40 ml**
REAGENT 3 Buffer 50 mmol/L, LiCl, pH 5.98	**1 x 40 ml**
COLUMNS Chromatographic columns	**20**

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 range between 20 and 28°C. Reagents and columns must have the same temperature before use.

PREPARATION OF HEMOLYSED SOLUTION

Pipette in a tube:

Blood	0.050 ml
Reagent 1	0.450 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.

CHROMATOGRAPHIC SEPARATION

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

Pipette into the column:

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

Pipette into the column:

Reagent 2	2.0 ml	discard the eluate

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

Reagent 3	2.0 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 tube (16 x 160 mm):

Hemolyzed solution	0.100 ml
Distilled water	12.0 ml

Mix and read the absorbance of total hemoglobin (A Hb TOTAL) 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 determine 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 local regulations.

REFERENCE

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

MANUFACTURER

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalogue number
	temperature limits
	use by
	caution
	read instructions for use

IVD CE

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