HbA1c HAEMOGLOBIN A1C MULTI PURPOSE REAGENT

KIT SPECIFICATIONS:

Cat. No.	Quantity	Reagent	Storage
GL2201HC	1 x 18 ml	R1A	2-8°C
	1 x 7.5 ml	R1B	
	1 x 11ml	R2	
	1 x 32 ml	Lysis Buffer	
GL2211HC	2 x 18 ml	R1A	
	2 x 7.5 ml	R1B	
	2 x 11ml	R2	
	2 x 32 ml	Lysis Buffer	

INTENDED USE:

In Vitro Diagnostic reagent pack for the quantitative determination of Haemoglobin A1c (HbA1c in %) in whole human blood on automated and semi automated Clinical Chemistry Analyser

SUMMARY AND EXPLANATION:

Glycosylated haemoglobin is formed by the combination of haemoglobin and glucose through a non-enzymatic reaction. The procedure is slow, permanent and irreversible, and lasts during the entire life cycle of a red blood cell, (approximately 120 days). The rate of glycosylated haemoglobin synthesis is proportional to blood glucose concentrations.; therefore, glycosylated haemoglobin, measured as a percent of total haemoglobin, reflects the mean blood glucose concentration over the previous 1-3 months. This therefore provides a suitable marker for the assessment of long term glycaemia in diabetic patients.

PRINCIPLE OF THE TEST:

Glenbio Direct Enzymatic HbA1c assay directly determines the % HbA1c in the sample and does not require an additional measurement of total haemoglobin. Samples are lysed and reacted with agents to eliminate signal interfering substances. The lysed whole blood then undergoes protease digestion, a process which releases amino acids from the haemoglobin beta chains. Glycated valines released in this process serve as substrates for specific recombinant fructosyl valine oxidase (FVO) enzyme. FVO specifically cleaves N-terminal valines, producing hydrogen peroxide, the rate of production of which can be measured spectrophotometrically.

WARNINGS AND PRECAUTIONS:

For In Vitro Diagnostics Use Only - For Professional Use Only
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.

Components Colour and Appearance:
Reagent 1a: Clear solution.
Reagent 1b: Pale yellow solution
Reagent 2: Pale yellow solution.
Haemolysis solution: Clear colourless liquid.
Any significant changes could indicate that the assay might be compromised. Refer to Laboratory's QC program for actions to be taken. In case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.

Safety precautions:
CAUTION: Take all necessary precautions required when handling laboratory reagents. Product is not hazardous under EU specifications.
Handling precautions:
- Take the necessary precautions required for handling all laboratory reagents.
- Do not use components past the expiry date stated on the Bottles.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Avoid contact with eyes and skin. If contacted, flush eyes and risen skin with a large amount of water. If irritation persists, consult with a physician.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Refer to local legal requirements for safe waste disposal.

INSTRUMENTS:

Instrument Applications are available on request

REAGENT PREPARATION AND STABILITY:

Reagents are all liquid ready to use.
Before use, mix reagent by gently inverting each bottle. Do not use the reagent described above for any other purpose that described herein.
This reagent kit is stable up to the expiry date stated on the label when stored at 2-8ºC.
For analysers capable of handling 3 reagents, R1A, R1B, R2 are ready to use. For analysers capable of handling only 2 reagents, HbA1c reagents R1A and R1B should be mixed in a 7:3 ratio and allowed to **sit at 2-8ºC for 24 hours prior to use**. To prepare sufficient R1AB mixture, pour the entire contents of R1B into R1A bottle. Mix gently by inversion.
Reconstituted R1AB thus prepared is stable for 4 weeks when stored at 2-8ºC. R1B and R2 reagents are light sensitive.

COMPONENT COMPOSITION:

Component	Ingredients
Reagent 1a	MES Buffer, Proteases, Triton-X-100, Redox agents
Reagent 1b	MES Buffer, Redox agents
Reagent 2	Tris Biuffer,FVO Enzyme, POD, Chromagen
Lysis Buffer	CHES Buffer, Triton-X-100 ,SDS, Redox reagents

TYPE OF SPECIMEN:

Sample Collection: Use whole blood treated with EDTA anticoagulant.
It is recommended to follow NCCLS procedures (or similar standardised conditions) regarding specimen handling. Specimen should be collected in an appropriate sample container, with proper specimen identification.
Stability of HbA1C in whole blood: Up to 2 Weeks at 2-8ºC.

Sample preparation:
Resuspend erythrocytes by gentle inversion(X5) prior to testing
Accuracy of the assay will be affected if whole blood is not thoroughly mixed prior to testing
Add 20µl of fully resuspended sample to 250 µl of Lysis Buffer. Mix gently using a pipettor without creating foam. Incubate at 25ºC for 10 minutes until red blood cells are completely lysed.
Complete lyses is observed when the mixture becomes a clear dark red solution without any particle matter
Incubate the sample for longer if necessary, to obtain complete lyses
The lysate will be stable for up to 4 hours at room temperature.
Calibrators and controls should be treated the same as patient samples prior to use.

TEST PROCEDURE:

Materials required but not supplied:

Description	Catalog. No.	Description	Catalog. No.
HbA1c Calibrator set	GL9708	Photometer	N/A
HbA1c Control Level Set	GL9104	General Laboratory Equipment	N/A

Assay procedure:
Wavelength: λ: 700 nm
Temperature: 37°C
Optical path: 1 cm light path.

	Calibrator	Sample
Reagent 1A	180 µl	180 µl
Reagent 1B	75 µl	75 µl
Sample		40 µl
Calibrator	40 µl	
Gently mix and Incubate at 37°C Measure the Optical Density (A1) after 4 minutes.		
Reagent 2	110µl	110 µl
Measure the Optical Density (A2) after 3 minutes. Calculate ΔAbs= A2-A1		

Use the calibration curve constructed to obtain % HbA1C from absorbance calculated

Calibration:
Using recommended Calibrators, calibrate the assay:
- Daily
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the photometer used.
- When Quality Control results are out of range.

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program:
Controls should be assayed:
- Prior reporting patient results.
- Following any maintenance procedure on the photometer used.
- At intervals established by the Laboratory QC Programme.

CALCULATION:

The analyser automatically calculates the HbA1c concentration in the sample.
The calibrator values are aligned with the DCCT system and are therefore reported in the NGSP[6] format.
IFCC values can be calculated using the following formula
NGSP = [0.915 x IFCC] + 2.15 [8,9]

EXPECTED VALUES:[3]

Normal 3.0 – 6.0% Controlled diabetics 6 – 9 %

Each laboratory should establish its own reference range. In using HbA1c to monitor diabetic patients, results should be interpreted individually.

PERFORMANCE CHARACTERISTICS:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Linearity:

The assay range is 4.0% - 12.0%

Interfering substances:

Ascorbic Acid	up to 12mg/dL
Bilirubin (Total)	up to 15 mg/dl
Bilirubin (Conjugate)	up to 13 mg/dl
Glucose	up to 4000mg/dL
Triglycerides	up to 4000mg/dL
Uric Acid	up to 30mg/dL
Urea	up to 80mg/dL

Acetylated, carbamylated and labile HbA1c do not adversely affect the Assay. Variant haemoglobin S, C and E do not significantly interfere with this assay

Precision:

Within Run N=20	Mean (%)	SD	% CV	Between Run N=5	Mean (%)	SD	% CV
Level 1	5.7	0.06	1	Level 1	5.7	0.10	1.8
Level 2	10.3	0.07	0.7	Level 2	10.3	0.18	1.8

Method Comparison:
A comparison with another commercially available HbA1c method gave the following results
$Y = 1.02 + 0.0135$, $r^2 = 0.9874$
44 samples were tested in the range 5 – 13%

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SYMBOLS:

The following symbols are used in the labelling of Glenbio systems:

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	Ab	Antibody
CAL	Calibrator	SUBS	Substrate
BUF	Buffer	CE	CE Mark - Device complies with the Directives 98/79/EC
	Storage temperature	→	Reconstitute with
	Expiry Date (Last day of the month)		Manufactured By
	Biological risk		Consult Instruction for Use

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