HbA1c Enzymatic **HBA1-201E**
Only for *in vitro* use in clinical laboratory
Store at 2-8ºC

HbA1c Enzymatic

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■ Principle of test
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 react 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.

■ Clinical significance
Diabetes Mellitus is a chronic disease characterized by a hyperglycemia. The consequences are metabolism disorders of carbohydrates, lipids and proteins. The risk of complications associated with diabetes, including nephropathy, retinopathy and cardiovascular diseases, increases in patients with poor metabolic control. In the diabetic patients, where blood glucose levels are elevated, HbAB1Bc is formed as a consequence of the non-enzymatic glycation of the N-terminus of the β-chain of haemoglobin molecule. The level of HbA1c is proportional to the level of glucose in the blood and has been widely accepted as an indicator of the mean daily blood glucose concentration over the preceding 6-8 weeks. It is therefore, a long-term indicator of diabetic control, whereas, the measurement of blood glucose is only a short-term indicator.

■ General 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 Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.

■ Kit components

Component	Ingredients	Concentration in Tests
Reagent 1a	MES Buffer, proteases, redox agents	15 mmol/l
Reagent 1b	Buffer, redox agents	60 mmol/L
Reagent 2	FVO Enzyme, POD, Chromogen	0.08 mg/dL
Lysis Buffer	CHES Buffer, detergent, redox reagents	0.05 mg/dL

■ Procedural Precautions
Reagent 1 and 2 and Haemolysis reagent are ready for use.
Before use, mix reagent by gently inverting each bottle.

If stored and handled properly at 2-8°C, the components are stable until the expiry date stated on the label.
Alterations in the physical appearance of the reagents or values of control materials outside of the manufacturer's acceptable range may be an indication of reagent instability.
Pre-mix R1a & R1b in the ratio 7:3 as required.
Depending on the number of tests required the entire bottle of R1b can be added to R1a. The mixture will be stable for 14 days when stored at 2-8°C or onboard the analyser.

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 2-8°C.

■ Usage and Dosage (assay procedures)
Test procedures
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 pipette without creating foam. Incubate at 25°C 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.

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 NGSP6 format. IFCC values can be calculated using the following formula: NGSP = [0.915 x IFCC] + 2.15 8,9

■ Reference Normal Values
3 - 6% and for controlled diabetics: 6 - 9%
Each laboratory should establish its own reference range to reflect the age, sex, diet and geographical location of the population.

■ Performances
1. Precision

Within Run	Mean (%)	SD	% CV	Between Run	Mean (%)	SD	% CV
Level 1	5.7	0.06	1	Level 1	5.7	0.1	1.8
Level 2	10.3	0.07	0.7	Level 2	10.3	0.1	1.8

2. Measuring range
4.0% – 16.0 %

3. 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

4. Method comparison
A comparison with another commercially available HbA1c method gave the following results:
Y = 1.02 + 0.0135, r2 = 0.9874
44 samples were tested in the range 5 – 13%

■ Precautions for Use or Handling
1. Safety warning
This product is intended for in vitro diagnostic use only and do not use in vivo.

2. Precautions for use
(1) Strictly follow the storage conditions for this kit.
(2) Do not use the reagents after the expiration data.
(3)The reagents in this kit are arranged to ensure accurate reaction.
Do not interchange reagents between different lots numbers.
(4) Do not use after the appearance is extraordinary.
(5) Please avoid contamination of microorganism.
Precaution for dispose
(1) Sample, liquid waste, and equipment should be disinfected with hypochlorite or autoclaved at 121℃ for least 1 hour.

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