BIOLABO REAGENTS
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HbA1c ENZYM

Enzymatic Method

Reagent for quantitative determination of HbA1c in human blood

REF 22050	**R1** 1 x 16 mL	**R2** 1 x 7 mL
	R3 1 x 10 mL	**R4** 1 x 40 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (2) (3) (4)

Reagents for the quantitative determination of HbA1c in human blood. HbA1c determination is commonly assayed for the evaluation of glycemic control in diabetes mellitus. HbA1c values provide an indication of glucose levels over the preceding 4-8 weeks. A higher HbA1c value indicates poorer glycemic control. Do not use for the diagnosis of diabetes mellitus.
The limitations of the method are known: they are related to a modified lifetime of red blood cells, physiological haemolysis or an insufficient level of total haemoglobin, which may invalidate the test result.

PRINCIPLE

HbA1c ENZYM test is an enzymatic assay in which lysed whole blood samples are subjected to extensive protease digestion with Bacillus *sp* protease. This process releases amino acids including glycated valines from the hemoglobin beta chains. Glycated valines then serve as substrates for specific recombinant fructosyl valine oxidase (FVO) enzyme, produced in *E. coli*. The recombinant FVO specifically cleaves N-terminal valines and produces hydrogen peroxide. This, in turn, is measured using a horseradish peroxidase (POD) catalyzed reaction and a suitable chromogen. No separate measurement for total Hemoglobin (Hb) is needed with this HbA1c ENZYM Test.
The HbA1c concentration is expressed directly as %HbA1c by use of a suitable calibration curve in which the calibrators have values for each level in %HbA1c

REAGENTS

Vial R1 **ENZYMES 1 BUFFER**

MES pH 7.0 5 mM
Proteases 4 KU/mL
Triton-X-100 0.5%
Redox agents >10mM

Vial R2 **REDOX BUFFER**

MES pH 6.3 1 mM
Redox agent <3 mM

VialR3 **ENZYMES 2 BUFFER**

Tris pH 8.0 15 mM
FVO enzyme >10 U/mL
POD 90 U/mL
Chromogen 0.8 mM

VialR4 **LYSING BUFFER**

CHES, pH 8.7 100 mM
Triton-X-100 1 %
SDS 0.45 %
Redox Agents 0.5 mM

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing.
- . Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

With 3 Reagents method : Liquid reagents contained in vial R1, R2, R3 and R4 are ready to use.

With 2 Reagents method: Prepare a working reagent by mixing 7 volumes of vial R1 with 3 volumes of vial R2 and let stand at 2-8°C overnight before use.

Mix gently by inversion before use

STABILITY AND STORAGE

Store at 2-8°C, well recapped in the original vial and away from light.

- Unopened, Reagents R1, R2, R3 and R4 are stable until expiry date stated on the label of the kit when stored and used as described.
- Once reconstituted, working reagent (R1+R2) is stable for 1 month when free from contamination.
- Discard any reagent if cloudy or if absorbance at 520 nm > 0.100.
- Don't use working reagent after expiry date stated on the label.
- R2 and R3 are light sensitive

SPECIMEN COLLECTION AND HANDLING (6)

Fresh venous blood collected with EDTA using aseptic technique. Special preparation of the patient is unnecessary. No special additives or preservatives other than anticoagulants are required.

Hemolysate preparation for patient(s), calibrators and controls:

1. Dispense 250 µL Lysing Buffer (vial R4) into well labelled plastic or glass test-tubes:
2. Add 20 µL of well mixed specimen (calibrators, controls, patient(s))
3. Mix well
4. Let stand for 10 min at room temperature until complete lysis is evident
5. Measure HbA1c (%) (§ **MANUAL PROCEDURE**)

If the test cannot be carried out on the same day, hemolysates may be stored up to 7 days at 2-8° C.

For longer storage, freeze specimen at -70° C for maximum 30 days.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. REF 22052, HbA1c ENZYM Standard Set
3. REF 22013, HbA1c Control Set.

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with

INTERFERENCES (5)

The assay is formulated for use with human whole blood in EDTA. Total hemoglobin in the sample should be in the range: 9- 21 g/dL

High HbF (>10%) may result in inaccurate HbA1c values.

The assay is not affected by the following interfering substances at the indicated concentrations:

Ascorbic acid 12 mg/dL, total bilirubin 15mg/dL, bilirubin (conjugated) 13mg/dL, glucose 4000mg/dL, triglyceride 4000mg/dl, uric acid 30 mg/dL, urea 80mg/dL.

The enzymatic reaction used in this assay (using stable glycated hemoglobin as substrate) is not adversely affected by acetylated, carbamylated and labile HbA1c.

Variant hemoglobin S, C and E do not significantly interfere with this enzymatic method.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

CALIBRATION

- HbA1c ENZYM Standard Set REF 22052 traceable to IFCC Reference Measurement Procedure for HbA1c.
- Or any calibrator traceable to a reference method or material.

Use as indicated in the insert (§ **MANUAL PROCEDURE**) to generate a reference curve.

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.

It is recommended to calibrate once a week

QUALITY CONTROL

- REF 22013: HbA1c Control Set
- Assayed control referring to the same method.
- External quality control program.
- Linearity of the assay should be verified with a commercial linearity check set or dilutions of a high specimen, at least every 6 months

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, patient's values from that run should not be reported and following actions should be applied:

1. Repeat the test with the same control.
2. If control is still out of range, use a new vial of control serum and repeat the test.
3. If control is still out of range, use a new vial of calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (7) (8) (9)

	HbA1c NGSP (%)	HbA1c IFCC (mmol/mol Hb)
Non-diabetic:	< 6.0 %	42
Glycemic control of a patient with diabetes:	< 7.0 %	53

In using Haemoglobin HbA1c to monitor diabetic patients should be interpreted individually. That is, the patient should be monitored against him or herself.

There is 3-4 week time lag before HbA1c reflects changes in blood glucose levels.

Each laboratory should verify the consistency of reference ranges for the population that it serves

LINEARITY

The assay is linear between 4.4% and 12%.

Samples with values above 12% should not be diluted and retested. Instead the values should be reported as higher than 12% (>12%).

PERFORMANCES CHARACTERISTICS

The performance characteristics for the HbA1c ENZYM Reagents were measured on Kenza 240TX.

The within run and between run precision studies was established by testing 2 control specimens following Valtech Protocol.

Within run N = 20	*Medium level*	*High level*
Mean %	6.6	8.9
S.D. %	0.12	0.12
C.V. %	1.8	1.3
Critères	< 3.8%	< 3.8%

Between run N = 19	*Medium level*	*High level*
Mean %	6.7	11
S.D. %	0.3	0.4
C.V. %	4.4	3.4
Critères	< 5%	< 5%

Detection limit: approximately 4.4%

Sensitivity for 1% HbA1c : approximately 0.004 Abs. at 700 nm.

Specificity: Monospecific

Comparison with an automated HPLC Procedure (Determined on Hitachi 917 chemistry analyzer)

The following HbA1c values were obtained using patient's whole blood as sample (n=44) : y = 1.0212 x +0.0135

Correlation coefficient 0.9874

Range of values 5% - 13% HbA1c

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature, away from light. Reconstitute calibrators and controls as indicated in the insert

Hemolysate Preparation: **Lyse patient's specimen, calibrators and controls** using reagent R4 as indicated in § "Specimen Collection and Handling"

Calibration curve: Use Standard Set REF 22052 (2 different levels) to generate a Calibration Curve.

Pipette into well identified test tubes:	Standards	Controls	Assays
Reagent R1	112 µL	112 µL	112 µL
Standards (2 different levels)	25 µL		
Controls		25µL	
Specimen			25 µL
Mix well. Let stand for 2 minutes at 37°C.			
Reagent R2	48 µL	48 µL	48 µL
Mix well. Let stand for 3 minutes at 37°C. Read absorbance A1 of lysed standards, controls and specimens at 700 nm against water			
Reagent R3	70 µL	70 µL	70 µL
Mix well. Incubate for exactly 3 minutes at 37°C. Read absorbance A2 of lysed standards, controls and specimens at 700 nm against water.			

Note: Application procedures with 2 reagents (using mixed R1/R2) or 3 reagents are available upon request.

CALCULATION (7) (8) (9)

The HbA1c concentration is expressed directly as %HbA1c by use of a suitable calibration curve using %HbA1c values of each calibrators.

Y(HbA1c %) = f (Abs A2-A1)

The values reported are aligned with the Diabetes Control and Complications Trials (DCCT) system and hence reported in the NGSP format. No calculation step is needed.

The relationship between HbA1c results from the NGSP network (%HbA1c) and the IFCC network (mmol/mol Hb) has been evaluated and a master equation has been developed:

NGSP = [0.09148 x IFCC] + 2.152.

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