

TurbiMAX HbA1c- Direct

Latex Enhanced Turbidimetric Immuno Assay (LETIA)

ORDERING INFORMATION

Ref. No.	Pack Size	Presentation
AVHBA1CT- 20	20 ml	(1 x 15 ml R1 + 1 x 5 ml R2)
AVHBA1CT- 40	40 ml	(2 x 15 ml R1 + 2 x 5 ml R2)
AVHBA1CT- 80	80 ml	(4 x 15 ml R1 + 4 x 5 ml R2)
AVHBA1CT- 100	100 ml	(2 x 37.5 ml R1 + 2 x 12.5 ml R2) With Four Calibrators Set

PRODUCT FEATURES

- Two Liquid Reagents (3 Parts R1+ 1 part R2).
- Latex Enhanced Turbidimetric Immuno Assay(LETIA)
- Linearity : 18.0 % NGSP
- Measuring wavelength 630 nm. (600-670)
- 10 Minutes Two Step - End Point Method (5 Minutes + 5 Minutes)
- Lyophilized 4 Level Calibrators Provided

INTENDED USE:

Kit is use for the quantitative determination of Hemoglobin A1c (HbA1c) in human blood. The determination of HbA1c is most commonly performed 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.

CLINICAL SIGNIFICANCE

Throughout the circulatory life of the red cell, Hemoglobin A1c is formed continuously by the addition of glucose to the N-terminal of the hemoglobin beta chain. This process, which is non-enzymatic, reflects the average exposure of hemoglobin to glucose over an extended period. In a classical study, Trivelli et al¹ showed that Hemoglobin A1c in diabetic subjects to be elevated 2-3 fold over the levels found in normal individuals. Several investigators have recommended that Hemoglobin A1c serves as an indicator of metabolic control of the diabetic patients, since Hemoglobin A1c levels approach normal values for diabetics in metabolic control. Hemoglobin A1c has been defined operationally as the "fast fraction" hemoglobins (HbA_{1a}, A_{1b}, A_{1c}) that elute first during column chromatography with cation-exchange resins. The non-glycosylated hemoglobin, which consists of the bulk of the hemoglobin has been designated HbA₀. The present procedure utilizes an antigen and antibody reaction to directly determine the concentration of the HbA1c.

PRINCIPLE

This method utilizes the interaction of antigen and antibody to directly determine the HbA1c in whole blood. Total hemoglobin and HbA1c have the same unspecific absorption rate to latex particles. When mouse antihuman HbA1c monoclonal antibody is added (R2), latex-HbA1c-mouse anti human HbA1c antibody complex is formed. Agglutination is formed when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA1c absorbed on to the surface of latex particles. The amount of agglutination is measured as absorbance. The HbA1c value is obtained from a calibration curve.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the labels when stored at 2-8°C and the contaminations is prevented during their use. Do not freeze the latex and diluent.

KIT COMPONENTS

- Latex Reagent R1
- Antibody Reagent R2
- Hemolysing Reagent R3
- HbA1c Calibrators (4) : Concentration as stated on the label

COMPOSITION

Latex Reagent R1: Latex 0.13%, Buffer, stabilizer.

Antibody Reagent R2: Mouse anti-human HbA1c monoclonal antibody 0.05mg/ml, goat anti-mouse IgG polyclonal antibody 0.08mg/dl, Buffer, stabilizers.

Hemolysing reagent R3: water and stabilizers.

REAGENT RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution.

When the reagent is stored properly at 2-8°C & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

Calibrator Preparation, Storage and Stability:

TurbiMAX HbA1c-Direct is provided with 4 Levels of Lyophilized Calibrators. Reconstitute each level with 1.0 ml of Distilled Water

Reconstituted Calibrators are stable for 15 Days once stored properly at 2-8°C. Aliquot it in to small volumes and store at 2-8°C for the contamination free use and for good reconstitution stability.

Reconstituted Calibrator must be added directly in to Reagent R1. It should not be further Lysed.

MATERIAL REQUIRED BUT NOT PROVIDED

Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

REAGENT DETERIORATION

Discard reagent if blank reagent absorbance exceeds 1.4 at 630 nm against distilled water.

WARNING & PRECAUTIONS

- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP

SPECIMEN COLLECTION & STORAGE

Special preparation of the patient is not necessary. Fasting specimens are not required. No special additives or preservatives other than anticoagulants are required. Collect venous blood with EDTA using aseptic technique. All human specimens should be regarded as potentially biohazardous. Therefore, universal precautions should be used in specimen handling (gloves, lab garments, avoid aerosol production, etc.).

Patient Blood Sample Hemolysate Preparation and Use:

To determine HbA1c, a hemolysate must be prepared for each patient sample. **Hemolysate should be prepared only for patient blood samples.**

- Dispense 1.0 ml Lysing Reagent into each tube labelling : Patient names. Plastic or glass tubes of appropriate size are acceptable.
- Place **20 µl** of well mixed Whole Blood into the appropriately labeled lysing reagent tube.
- Allow to stand for 10 minutes or until complete lysis is evident. Hemolysates may be stored up to 7 Hours at 2-8°C

SYSTEM PARAMETERS

Reaction Type (Mode)	End Point- Non Linear- Multi Standard
Reaction Direction	Increasing
Wave Length	630 nm (600-670 nms)
Flow Cell Temp.	37°C
Blank	Distilled Water Blank
Reagent Volume	360 µl (R1) + 120 µl (R2)
Lysate of Control/Blood } Sample Volume	20 µl
Calibrator Concentration	(Printed on the Vials Label)
Linearity	18.0%
Mix and incubate for 5 minutes at 37 °C and read absorbance (A) at 630 nms (600-670 nms)	

Reagent	Calibrator	Sample/Control
HbA1c R1	360 µl	360 µl
Reconstituted Calibrators 1,2,3,4	20 µl	----
Hemolysate of the Whole Blood	----	20 µl
Mix and Incubate for 5 Minutes at 37 °C		
HbA1c R2	120 µl	120 µl

Calculations with Calibrators/ Calibration Curve/ Result Interpretation:

CALCULATION

Plot the absorbances of all the Calibrators versus their respective concentrations (HbA1C%) on a non linear graph paper. HbA1c Results according to NGSP for the samples and controls are determined using the prepared calibration curve.

HbA1c % in the sample is calculated by interpolation of Abs of Sample on the calibration curve.

For calculation of results according to IFCC use IFCC calibrator values (See Calibrator Insert) or use following equation:

Note: Please note that the NGSP Units (%) and IFCC Units (mmol / mol Hb) are listed in the Calibrator Insert given separately:

Unit Conversion:

NGSP= (0.0915 x IFCC) +2.15

The relationship between eAG (Estimated Average Glucose) and HbA1c:

$eAG (mg/dL) = (28.7 \times HbA1c) - 46.7$

EXPECTED VALUES:

Recommended Values:

Less than 6% for a non-diabetic,

Less than 7% for glycemic control of a person with diabetes.

Each laboratory should establish its own expected values. In using Hemoglobin A1c to monitor diabetic patients, results should be interpreted individually. That is, the patient should be monitored against him or herself. There is a 3-4 week time before Hemoglobin A1c reflects changes in blood glucose level.

QUALITY CONTROL & CALIBRATION

HbA1C Controls are recommended for daily quality control. The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits

PERFORMANCE CHARACTERISTICS:

1. Linearity

Linearity : 18% NGSP

2. Sensitivity/ Limit of Detection (LOD)

The lower limit of detection is 2.0 % NGSP

3. Interferences:

Hemoglobin (10 g/l), bilirubin (40 mg/dl) and lipemia (10 g/l) do not interfere.

4. Precision: The reagent has been tested for 20 days, using two levels of serum in a EP5-based study (NCCLS).

Intra-Assay

N=20	Mean (% NGSP)	SD (% NGSP)	CV%
Control 1	5.48	0.078	1.43
Control 2	10.28	0.176	2.68

Inter-Assay

N=20	Mean (% NGSP)	SD (% NGSP)	CV%
Control 1	5.49	0.15	2.73
Control 2	10.32	0.275	2.66

5. Method Comparison:

A study using 40 human specimens between this Hemoglobin A1c procedure and an automated HPLC procedure (Tosoh) yielded a correlation coefficient of 0.988 and a linear regression equation of $y=1.050x - 0.481$. (Syx = 0.332)

LIMITATIONS (calibration curve): 2-18 % NGSP, under the described assay conditions. Samples with higher concentrations should be diluted 1/5 in saline (10 parts serum sample + 40 parts normal saline ex: 10µl serum sample+40 µl saline) and retested again and the results should be multiplied by 5. The linearity limit and measurement range depends on the sample to reagent/ratio, as well as the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.














WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.

REFERENCE

1. Clinical Laboratory Diagnostics., Lothar Thomas, MD. 1st Ed., 1998, TH Books Verlagsgesellschaft mbH, Frankfurt, Germany, pgs 142-148.
2. IFCC reference system for measurement of hemoglobin A1c in human blood and the National Standardization Schemes in the United states, Japan and Sweden: a method comparison study, Clinical chemistry (2004) 50:1, pgs 166-174.
3. Eric S Kilpatrick, J Clin Pathol 2000; 53, pgs 335-339.
4. H. B. Chandalia and P. R. Krishnaswamy, Glycated Hemoglobin, Current Science Vol. 83, No. 12, 25 December 2002.
5. David M.N et.al, Management of Hyperglycemia in Type 2, A consensus algorithm for the initiation and adjustment of therapy, Diabetes Care, Vol. 29, Number 8, August 2006, pgs 1963-1972.

Symbols Used on Pack

	Catalogue Number		Warning/Caution
	Batch No.		In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
	Contains sufficient no. of test		

Ver. : 05/12-25