

Kit for the quantitative determination of Hemoglobin A1c in Human Blood

### Intended 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.

This product is for in vitro diagnostic use only.

### Summary and Explanation of Test:

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 al<sup>1</sup> 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<sub>1a</sub>, A<sub>1b</sub>, A<sub>1c</sub>) 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 HbA<sub>0</sub>. 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.

### Reagents

Reagent 1: Latex 0.13%, Buffer, stabilizer.

Reagent 2: Mouse anti-human HbA1c monoclonal antibody 0.05mg/ml, goat anti-mouse IgG polyclonal antibody 0.08mg/dl, Buffer, stabilizers.

Hemolysis reagent: water and stabilizers.

### Reagent Storage

Store all reagents refrigerated at 2-8°C.

### Reagent Preparation

R1, R2 and Hemolysis reagents are supplied as ready to use liquids. Mix gently before use.

### Reagent Deterioration

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.

### Instruments

Refer to specific instrument application for suggested settings.

### Precautions

1. This reagent is for in vitro diagnostic use only.
2. Not for internal or external use in humans or animals.

### Specimen Collection and Preparation:

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.).

### Test Procedure:

To determine HbA1c, a hemolysate must be prepared for each sample:

1. Dispense 1ml Hemolysis Reagent into tubes labeled: Calibrators, Controls, Patient Blood Samples, etc. Note: Plastic or glass tubes of appropriate size are acceptable.
2. Place **20 µl** of well mixed Whole Blood/ Reconstituted Calibrator/ Reconstituted Control into the appropriately labeled lyse reagent tube. Mix.
3. Allow to stand for 10 minutes or until complete lysis is evident. Hemolysates may be stored up to 7 Hours at 2-8°C

Follow the same procedure for Calibrators and Controls

### Assay Procedure: (Multi Point Calibration with 2 Calibrator Levels and 4 Calibrator Levels)

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 Calibrator / Control/ Blood Sample Volume)	20 µl
Calibrator Concentration	— (On the Vials Lot Specific)
Linearity	17.0%

Procedure:

Reagent	Calibrator	Sample/Control
HbA1c R1	360 µl	360 µl
Hemolysate of the Calibrator	20 µl	----
Hemolysate of the Whole Blood	----	20 µl
<b>Mix and Incubate for 5 Minutes at 37 °C</b>		
HbA1c R2	120 µl	120 µl

Mix and incubate for 5 minutes at 37 °C and read absorbance (A) at 630 nms (600-670 nms)

Calculations with Calibrators/ Calibration Curve/ Result Interpretation:

Calculate the Absorbance of Calibrators

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:

Less than 6.0 % for Non-Diabetics  
6.0 to 6.4% Risk of developing Diabetes  
More than or Equal to 6.5%: Diabetes

### HbA1c goals in treatment of Diabetes:

Good Control: 6.0 - 7.0%  
Fair Control: 7.0 - 8.0%  
Poor Control : More than 8.0%

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.

### Performance:

- Linearity:** The Hemoglobin A1c assay range is 2.0 % - 17.0 % NGSP
- 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  $y=1.050x - 0.481$ . ( $Syx = 0.332$ )

### 3. Precision:

**Within Run:** The within run precision was established by assaying two blood samples following NCCLS protocol EP5 on a Hitachi 917.

Level	Mean	Std. Dev.	% C.V.
Low	5.48	0.078	1.43
High	10.28	0.176	1.72

**Day to Day:** The between day precision was established by assaying two blood samples following NCCLS protocol EP5 on a Hitachi 917.

Level	Mean	Std. Dev.	% C.V.
Low	5.48	0.152	2.77
High	10.28	0.275	2.68

**4. Sensitivity:** Sensitivity was investigated by reading the change in absorbance at 630 nm for a saline sample and a whole blood sample with a known concentration. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used (Hitachi 717), the HbA1c reagent showed little or no drift on the zero sample.
















### Ordering Information

Ref./Cat. No.	Pack Size	Presentation
P-HGB(A1C) - 20	20 ml	(15 ml R1 + 5 ml R2) with Two Calibrators Set & Four Calibrators Set
P-HGB(A1C) - 40	40 ml	(2 x 15 ml R1 + 2 x 5 ml R2) with Two Calibrators Set & Four Calibrators Set

### Product Features

- Two Liquid Reagents (3 Parts R1+ 1 part R2).
- Latex Enhanced Turbidimetric Immuno Assay(LETIA)
- Linearity : 17.0 % NGSP
- Measuring wavelength 630 nm. (600-670)
- 10 Minutes Two Step - End Point Method ( 5 Minutes + 5 Minutes)
- Lyophilized Calibrators Separately Provided
- Available in 4 Level Calibrator format and 2 Level Calibrator Format

Symbols used with IVD devices

	Date of manufacture		Manufactured by
	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)		Reagent
	Calibrator Material		Batch code
	Temperature limitation (store at)		Control
	Consult instructions for use		Keep dry Keep away from rain
	Catalog Number		

eIFU Indicator



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SCAN TO EXPLORE MORE

Manufactured in India by :  
Pariksha Biotech Pvt Ltd,  
Plot no.1/B-14, SVICE,  
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