

# TURBICHEM HbA1C

(Turbilatex method)



KIT NAME	KIT SIZE	CAT. NO
Turbichem - HbA1C	1 x 40 ml	TA1C01040M

## INTRODUCTION

Glycated Hemoglobin A1c (HbA1c) is intended for Invitro quantitative determination of HbA1c in human whole blood. Hemoglobin A1c is a subtype of hemoglobin A that is formed by a non-enzymatic process that adducts glucose to the N-terminal of the hemoglobin beta chain. This process reflects the average hemoglobin exposure to glucose over an extended period and provides clinical significance in monitoring the blood glucose level. Studies have shown HbA1c in diabetic patients to be 2-3 times the levels found in normal individuals. HbA1c can be used as an indicator of metabolic control of the diabetic.

## METHOD PRINCIPLE

The kit utilizes latex-enhanced immunoturbidimetry to measure the HbA1c level in human whole blood. The Kit utilizes the antibody-antigen reaction to directly measure the HbA1c level in whole blood. The first reaction, occurring after the sample is mixed with R1, consists of unspecified binding of total hemoglobin and HbA1c to the latex particles at the same rate. The second reaction occurs after the addition of R2 that contains mouse anti-human monoclonal antibody and goat anti-mouse IgG polyclonal antibody. Agglutination complexes will be formed from the interaction of the HbA1c bound to the latex particles with the respective antibodies. The agglutination can be measured as an absorbance which is proportional to the amount of HbA1c bound to the latex, and because the total hemoglobin and HbA1c bind to the latex at the same rate, the % HbA1c in total hemoglobin can be obtained from a calibration curve.

## KIT CONTENTS

R1 - HbA1c Latex	1 x 30 ml
R2 - HbA1c Buffer	1 x 10 ml
R3 - Lysing Reagent	1 x 50 ml
HbA1c Calibrator Set	4 vials

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 10 days on board the analyser at 2-10°C. Protect from light and avoid contamination.

The calibrators are lyophilized form contains 4 levels, the concentration and reconstitution instructions to be followed as per the volume mentioned on the calibrator vial labels.

## WORKING REAGENT PREPARATION AND STABILITY

Assay can be performed with use of separate R1-HbA1c and R2-HbA1c reagents or with use of working reagent. For working reagent preparation mix gently 3 parts of R1-HbA1c with 1 part of R2-HbA1c. Avoid foaming.

Stability of working reagent : 2 days at 2-8°C

## CONCENTRATIONS IN THE TEST

- R1 - Latex 0.1%; Glycine buffer, pH 3.0, 15mmol/L
- R2 - goat anti-mouse IgG polyclonal antibody 0.8mg/dl; Mouse anti-human HbA1c monoclonal antibody 0.05 mg/ml; Glycine buffer, pH 3.0, 60mmol/L
- R3 - H2O2, stabilizers

## WARNINGS AND NOTES

1. The Kit is for in vitro diagnostic use only. Not for use in humans or animals.

2. The instructions must be followed to obtain accurate results.
3. Do not use the reagents beyond the expiration date.
4. Treat all specimens as infectious. Proper handling and disposal procedures of specimens and test materials should be strictly followed.

## ADDITIONAL EQUIPMENT

- Automatic analyzer or photometer able to read at 630 nm
- Thermostat at 37°C
- General laboratory equipment

## SPECIMEN

The Test can be performed with human blood without special preparation of the patient. Follow standard laboratory procedures to collect specimens with EDTA.

## PLOTTING OF MULTIPOINT CURVE

The Turbichem HbA1c is based on Non-Linear Reactions, hence it is strongly recommended to run Multi-standard mode to plot the Multi-point curve to have better accuracy and precise result.

## PROCEDURE

These reagents may be used both for manual assay (Sample Start and Reagent Start method) and in several automatic analyzers. Applications for them are available on request.

Wavelength	630 nm
Temperature	37°C
Cuvette	1 cm

## HEMOSYLATE - Step One: ( Only for Blood Sample)

1. Mix 500 µl of Hemolysis Reagent with 10 µl of well mixed whole blood for Test (T)
2. Wait for 5 minutes or until complete lysis is evident before using the sample.
3. If immediate testing is not possible, hemolysates may be stored up to 10 days at 2-8°C.

Reagent	Calibrator (C)	Test (T)
R1 HbA1C Latex	750 µl	750 µl
Lysafe Sample from Step 1	-	20 µl
Direct Calibrator (No need lysate)	20 µl	-
Bring upto the temperature of determination. Then add		
R2 HbA1C Buffer	250 µl	250 µl

Mix well, after about 10 sec. (37°C) read the absorbance A1 of the test (T) and calibrator (C) against air or water. After exactly 300 secs. (for all temperature) read the absorbance A2 of the test (T) and calibrator (C). Calculate  $\Delta A/\text{min}$ . (A2 - A1) for the test and calibrator.

## CALCULATION

HbA1c concentration =  $\Delta A(T) / \Delta A(C) \times \text{calibrator concentration}$

## REFERENCE VALUES

HbA1c in whole blood	< 6%	Non-diabetic
	6 to 7%	Glycemic Control
	7 to 8%	Fair Control
	> 8%	Poor Control

It is recommended for each laboratory to establish its own reference ranges for local population.

## QUALITY CONTROL

To ensure adequate quality control, each run should include assured normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls

## PERFORMANCE CHARACTERISTICS

- **Linearity:** 2% to 18%
- **Precision:** within Run  $CV \leq 5\%$
- **Specificity / Interferences**  
No interference detected for bilirubin upto 0.5 g/L ascorbic acid 0.5 g/L, triglycerides 20 g/L, carbamylated Hb 7 mmol/L and acetylated Hb 5.0 mmol/L 5

## WASTE MANAGEMENT

Please refer to local legal requirements.

## LITERATURE

1. American Diabetes Association: Clinical Practice Recommendations (Position Statement). Diabetes Care 24 (Suppl 1): S33-S55, (2001).
2. Bates, H.M., Lab. Mang., Vol 16 (Jan. 1978).
3. Ceriello, A., et al, Diabetologia 22, p. 379 (1982).
4. Engbaek, F., et al, Clin. Chem. 35, pp. 93-97 (1989).
5. Fluckiger, R., et al, New Eng. J. Med. 304 pp. 823-827 (1981).
6. Gabbay, K.H., Hasty, K., Breslow, J.L., Ellison, R.C., Bunn, H.F., and Gallop, P.M., J. Clin. Endocrinol. Metab. 44, 859 (1977).
7. Gonen, B., and Rubenstein, A.H., Diabetologia 15, 1 (1978).
8. Little, R.R., et al, Clin. Chem. 32, pp. 358-360 (1986).
9. Nathan, D.M., et al, Clin. Chem. 29, pp. 466-469 (1983).
10. Tietz, N.W., Textbook of Clinical Chemistry, Philadelphia, W.B.unders Company, p.794-795 (1999).
11. Trivelli, L.A., Ranney, H.M., and Lai, H.T., New Eng. J. Med. 284, 353 (1971).

## SYSTEM PARAMETERS

Method	Fixed Time (2-Point)	End Point
Wavelength	630 nm	630 nm
Zero Setting	Distilled Water	Distilled Water
Temperature Setting	37° C	37° C
Incubation Temperature	37° C	37° C
Incubation Time	----	5 mins + 5 mins
Delay Time	10 secs	----
Read Time	300 secs	----
No. of Reading	2	2
Interval Time	----	----
Sample Volume	0.02 ml (20 ul)	0.02 ml (20 ul)
Reagent Volume	1.0 ml (1000 ul)	1.0 ml (1000 ul)
Standard Concentration	Refer Calibrator Vial	Refer Calibrator Vial
Units	%	%
Factor	----	----
Reaction Slope	Increasing	Increasing
Linearity	18%	18%



## Genuine Biosystem Private Limited

Plot No.97 & 98, kattabomman street,  
Parvathy Nagar Extension,  
Old Perungalathur, Chennai - 600063, India.  
Ph: +91-44-48681845  
Email: genuinebiosystem@gmail.com  
website: www.genuinebiosystem.com