Hemoglobin A1c (HbA1c) Turbidimetric Immunoassay Kit

Catalogue number: 51A1c

For the quantitative determination of HbA1c
in human blood

This package insert must be read in its entirety before using this product
Use only the current version of product data sheet enclosed with the kit

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FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

Version: 5.22

TABLE OF CONTENT

PACKING SPECIFICATION

Cat. No.	Size	Hemolysis reagent	Approximately tests
51A1c-05	R1: 15ml, R2: 5ml	50ml	100
51A1c -10	R1: 30ml, R2: 10ml	100ml	200
51A1c -20	R1: 60ml, R2: 20ml	200ml	400
51A1c -50	R1: 150ml, R2: 50ml	500ml	1000
51A1c -100	R1: 300ml, R2: 100ml	1000ml	2000

INTRODUCTION

HbA1c is the term known as glycated hemoglobin. Throughout the whole life cycle of red blood cell, HbA1c is formed continuously by the adduction of glucose to the N-terminal of the hemoglobin beta chain. Formation of the sugar-Hb linkage indicates the presence of excessive sugar in the bloodstream, often indicative of diabetes. Therefore, measurement of %HbA1c can also be used to determine three-month average blood sugar level since the average lifespan of a red blood cell is four months. HbA1c level in diabetic subjects will be elevated by 2-3 fold over the levels found in normal individuals. The cut-off value for %HbA1c is 6.5%. The higher the %HbA1c, the higher chance the diabetic subjects are going to develop complications.

HbA1c PETIA kit developed by IMD can accurately measure %HbA1c in human whole blood sample.

PRINCIPLE OF THE ASSAY

This assay is a turbidimetric immunoassay for the quantitative measurement of %HbA1c in human whole blood. A standard or sample is added into a cuvette and mixed with the reaction buffer R1. Total hemoglobin and HbA1c have the same unspecific absorption rate to latex particles. After a short incubation, the test reagent R2 is added into the cuvette and mixed. 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 extent to which the microparticles aggregate is quantified by the amount of light scattering measured as absorbance by a chemistry analyzer. The %HbA1c in unknown samples can be interpolated from a reference curve using the standards provided.

REAGENTS SUPPLIED

R1 – A ready-to-use suspension of latex microparticles

R2 – A ready-to-use mixture of mouse anti-human HbA1c monoclonal antibody and goat anti-mouse IgG

Hemolysis reagent – Water and hemolysis components

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Clinical chemistry analyzer
2. HbA1c calibrator, provided separately (Cat No: 51A1c -S1)
3. HbA1c quality controls, optional, provided separately (51A1c -C1)
4. Deionized water
5. Analyzer-specific reagent containers for R1 and R2

STORAGE

The kit should be stored at 2-8°C upon receipt. Once opened, the reagents may be stored at 2- 8°C for up to 4 weeks.

SAMLE HANDLING

This kit can be used to determine %HbA1c in human whole blood samples. Blood specimens should be collected aseptically into EDTA collection tubes. 5 µL of blood samples are mixed with 500 µL of hemolysis reagent. Mix gently up-and-down. Allow to stand for 5 minutes or until complete lysis is evident. Avoid vertexing or centrifugation of the samples. Hemolysates may be stored up to 10 days at 2-8°C. 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.).
For STD re-constitution, add 500 µL of ddH_2O. Mix gently up-and-down. Allow to stand for 5 minutes. Avoid vertexing or centrifugation.

ASSAY PROCEDURE

Assay procedures may vary depending on the automated chemistry analyzer to be used. A general example of assay procedures is stated as follow:

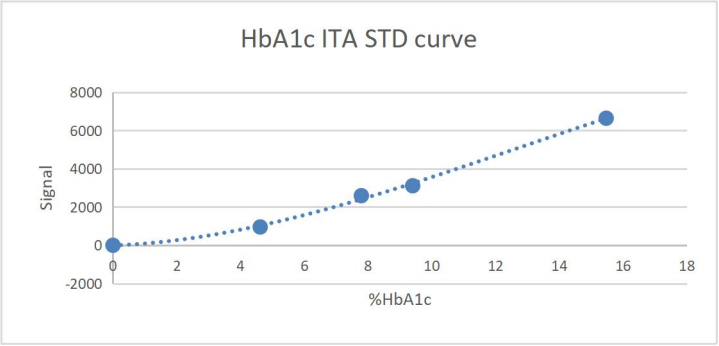
1. Dispense 150µl of R1 into a clean cuvette
2. Add 4µl of sample and incubate at 37˚C for 5 minutes
3. Further add 50µl of R2
4. Read change of absorbance at 660 nm for 5 - 8 minutes after the addition of R2
5. Calculate the %HbA1c in unknown sample by interpolation from a reference curve using the standards provided

Caution: Different lot of R1, R2, standard and control should not be mixed for use

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only (%HbA1c may vary slightly for different lot of standard). A standard curve should be generated for each assay.

%HbA1c	Absorbance (660 nm)
0	-0.0001
4.618	0.0955
7.79	0.2591
9.401	0.3110
15.469	0.6638



CALCULATION

1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against %HbA1c (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. 4-parameter curve fitting can be used for calculation.
3. Determine %HbA1c of samples from standard curve.

ASSAY CHARACTERISTICS

A. Sensitivity

The sensitivity is defined as the lower limit of detection and is estimated as the mean of the blank sample plus three times the SD obtained from the blank sample. The sensitivity of HbA1c assay is 2%

B. Precision

The precision of the HbA1c assay is CV < 3%. Three whole blood samples were assayed 20 times separately.

Sample	Mean (%HbA1c)	SD (%HbA1c)	CV
Sample 1	5.71	0.041	0.71%
Sample 2	6.16	0.122	1.98%
Sample 3	8.23	0.190	2.30%

C. Linearity

The HbA1c assay is linear between 2% to 16%.

D. Traceability

Our kit has been traced by comparison to BioRad HbA1c HPLC kit. The recovery ranges from 99% to 102% with a recovery of 99.9%.

IMD ITA (%HbA1c)	BioRad HPLC (%HbA1c)	Recovery
4.757	4.8	99%
5.444	5.4	101%
6.098	6.0	102%
6.594	6.5	101%
8.080	8.1	100%
8.844	8.7	102%
9.272	9.4	99%
13.427	13.3	101%
	Correlation	99.9%

E. Interference

No interference was detected with carbamylated hemoglobin up to 5 g/L, conjugated bilirubin up to 300 mg/L, free bilirubin up to 300 mg/L, and up to 5g/L lipid emulsion.

References

1. American Diabetes Association: Clinical Practice Recommendations (Position Statement) Diabetes Care 24 (Suppl. 1): S33-S55 (2001).
2. Campbell L, Pepper T, Shipman K. J Clin Pathol 2019;72:12–19.
3. Gonen, B., and Rubenstein, A.H., Diabetologia 15, 1 (1978).

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