



**INTENDED USE**

The TRUEchemie Glycosylated Hemoglobin (HbA1c) Test Kit (Immunoturbidimetry) liquid reagent is used for the direct quantitative determination of percent HbA1c (hemoglobin fraction) in human whole blood.

**INTRODUCTION**

Normal levels of glucose produce a normal amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. The test is limited to a three-month average because the lifespan of a red blood cell is four months (120 days). However, since RBCs do not all undergo lysis at the same time, HbA1c is taken as a limited measure of 3 months. In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, neuropathy, and retinopathy.

**PRINCIPLE**

The percentage of HbA1c in whole blood can be directly determined by utilizing the interaction of antigen and antibody. Total hemoglobin and HbA1c have the same unspecific absorption rate to Latex particles. When we add mouse antihuman HbA1c monoclonal antibody (containing Multiple subunits), "Latex-HbA1c-mouse antihuman HbA1c monoclonal antibody-HbA1c-Latex" complex is formed. The amount of this complex 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.

**PACK SIZE**

Kit Size	20 ml (1x20 ml)	40 ml (2x20 ml)	20 ml (1x20 ml)	40 ml (2x20 ml)	(4x0.5 ml)
Cat No.	ADX946	ADX947	ADX943	ADX944	ADX948
Kit Contents					
1) HbA1c Reagent (R1)	1 x 15 ml	2 x 15 ml	1 x 15 ml	2 x 15 ml	--
2) HbA1c Reagent (R2)	1 x 5 ml	2 x 5 ml	1 x 5 ml	2 x 5 ml	--
3) HbA1c Reagent (R3)	1 x 25 ml	2 x 25 ml	1 x 25 ml	2 x 25 ml	--
4) HbA1c Calibrators	4 x 0.5 ml	4 x 0.5 ml	--	--	4 x 0.5 ml

**REAGENTS COMPOSITION**

HbA1c Reagent (R1) : Latex 0.13%, Buffer, stabilizer Sodium azide (0.95 g/L)

HbA1c Reagent (R2) : Buffer, Mouse anti-human HbA1c monoclonal antibody: 0.05 mg/mL, goat anti-mouse IgG polyclonal antibody 0.08 mg/dL., Stabilizers

HbA1c Reagent (R3) : Hemolysing reagent: Water and stabilizers.

**REAGENT PREPARATION**

Ready to use reagents.

**WARNINGS AND PRECAUTIONS**

- For in vitro diagnostic use.
- Specimens should be considered infectious and handled appropriately.
- Volume of reagents and samples can be adjusted as per the instrument but the ratio of the reagent be the same
- If the reagents became turbid or the absorbance of blank reagent is higher than 1.000, it means that the reagent is invalid and you should discard it.
- The disposal of the residues has to be done as per local legal regulations.

**CALIBRATION**

When using TRUEchemie HbA1c calibrators to do the multi-point calibration, the calibration cycle is 14 days. Re-calibration is required when changing reagent batch. It is recommended to use the normal & pathological quality control as internal quality control. Corrective action shall be done if controls do not recover within the acceptable tolerance.

**REAGENT STORAGE & STABILITY**

The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2-8°C. Do not use reagents over the expiration date.

**REAGENTS : DO NOT FREEZE**

Calibrators: Reconstituted calibrators are stable for 20 days at 2-8°C .

**SAMPLE / SPECIMEN AND STORAGE**

Whole Blood samples are stable for 5 days at 2-8°C.

**CALIBRATOR PREPARATION**

The kit is packed with 4 calibrators (cal - 2 to cal - 5). Calibrator - 1 will be distilled water. Calibrator - 2 to Calibrator - 5 preparation as given below:

- Reconstitute the calibrator with 0.5 ml DI water.
- Mix thoroughly by gentle vortexing for 30 seconds.
- Stand for 30 minutes at room temperature and protect from light. Meanwhile, shake and flip over the brown bottle gently to ensure the content is fully dissolved. Avoid air bubbles.

**Lysing of calibrator:**

- Dispense 0.5 ml (500 µl) of DI water into a tube
- Add 0.015 ml (15 µl) of well mixed reconstituted calibrators.
- Mix thoroughly by gentle vortexing for 30 seconds
- Stand for 10 -15 minutes at room temperature until complete lysis.(Don't exceed above the given time)
- Take 0.008ml ( 8 µl) of above lysate for testing.

**SAMPLE PREPARATION**

- Only below samples were tested and found suitable for use:  
Dipotassium EDTA(K2-EDTA)  
Lithium Heparin(Li-Heparin)
- Do not use samples that are heat-inactivated, pooled or with obvious microbial contamination.
- Fresh/Non-Frozen samples: Do not centrifuge fresh/Non-frozen samples. Samples must be mixed thoroughly prior to use.

**4. HEMOLYSATE PREPARATION:**

- To determine the level of HbA1c, a hemolysate must be prepared for each sample:
- Dispense 0.500 ml (500 µl) of Hemolyzing Solution (HbA1c Reagent -3 (R3)) into a tube.
  - Add 0.010 ml (10 µl) of well mixed whole blood sample.
  - Mix thoroughly by gentle vortexing for 30 seconds
  - Stand for 2 minutes at room temperature until complete lysis.
  - Take 0.008 ml (8 µl) of above lysate for testing.

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Pipettes to accurately measure required volumes.
- Test tubes/rack
- Timer
- 37°C heating block or water bath
- Photometer capable of accurately measuring absorbance at 660 nm

**TEST PROCEDURE FOR PREPARATION OF CALIBRATION CURVE**

Wavelength : 660 nm  
Temperature : 37 °C  
Prewarm the Reagent to reaction temperature.

Reagent	Cal -1 (DI Water)	Cal -2	Cal -3	Cal -4	Cal -5
HbA1c Reagent (R1) (µl)	300	300	300	300	300
Calibrator (µl)	8	8	8	8	8

Mix well and incubate for 5 min at 37 °C

HbA1c Reagent (R2) (µl)	100	100	100	100	100
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Mix well, incubate 5 min. at 37 °C and read the absorbance of blank, calibrators at 660 nm.

**Calculations :**

**Δ O.D of Calibrator = O.D Calibrator-O.D Blank.**

Plot the Δ O.D of each calibrator versus assigned concentration (HbA1c%) on a non-linear graph.

**TEST PROCEDURE FOR PREPARATION OF SPECIMEN**

Wavelength : 660 nm  
Temperature : 37 °C  
Prewarm the Reagent to reaction temperature.

	Blank (µl)	Sample (µl)
HbA1c Reagent (R1)	300	300
Sample	--	8
Distilled Water	8	--

Mix well and incubate for 5 min at 37 °C

HbA1c Reagent (R2)	100	100
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Mix well, incubate 5 min. at 37 °C and read the absorbance of blank, samples at 660 nm.

**Calculations:**

**Δ O.D of Sample = O.D Sample - O.D Blank** HbA1c % in the sample is calculated by interpolation of OD of sample on the calibration curve.

The TRUEchemie HbA1c assay is traceable to the International Federation of Clinical Chemistry (IFCC) reference standard.

The default result unit for the assay is % HbA1c. For alternative units, manual calculations can be used according to below equations:

$$\text{NGSP \% HbA1c to IFCC mmol/mol: } [\% \text{HbA1c} \times 10.93] - 23.50$$

$$\text{IFCC mmol/mol to NGSP \%HbA1c: } \text{mmol/mol} \times 0.09148 ] + 2.152$$

HbA1c results are calculated from a spline data reduction method to generate a calibration curve.

**QUALITY CONTROL**

Control Sera are recommended to monitor the performance of automated assay procedures. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

**EXPECTED VALUE**

3.8%~5.8%(Varies as per different places)  
Each laboratory should establish its own range of reference values.

HbA1c Value	Glycaemic Goal
< 8 % HbA1c	Less Stringent
< 7 % HbA1c	General (Non-Pregnant Adults)
< 6.5 % HbA1c	More Stringent

It is strongly recommended that glycemic goals are individualized following current professional society recommendations

**PERFORMANCE CHARACTERISTICS**

Sensitivity: 2%  
Linearity: Highest Calibrator Value.  
Sample above the measuring range should not be diluted and retested. These samples should be tested with alternative methods. The linearity limit depends on the sample / reagent ratio, as well as the analyzers used.

**PRECISION:**

Intra-assay precision within run (n=10)	Mean (%)	SD (%)	CV (%)
Low	5.56	0.10	1.77
High	11.64	0.12	1.0

Inter-assay precision run to run (n=10)	Mean (%)	SD (%)	CV (%)
Low	5.53	0.17	3.08
High	11.60	0.09	0.80

The reagent was tested for 10 days, using two different HbA1c concentrations. The coefficient of variation was <5%.

**AUTOMATED PROCEDURE**

Appropriate Program sheet is available for different analyzers upon request.

**METHOD COMPARISON**

Results obtained using TRUEchemie Hb1Ac reagent (y) did not show systematic differences when compared with another commercial reagent (x) with similar characteristics. The results obtained is below: The correlation coefficient (r<sup>2</sup>) was 0.993 and the regression equation is y=0.980x+0.1383. The results of the performance characteristics depend on the analyzer used.

**INTERFERENCES**

The assay is not affected by the following interfering substances at the indicated concentrations:  
 Bilirubin up to 688 µmol/L no interference effect  
 Triglyceride up to 11.3 mmol/L no interference effect  
 Ascorbic acid up to 0.5 g/L no interference effect

**WASTE MANAGEMENT**

Please refer to local regulation requirements.















**SYSTEMS PARAMETERS**


Mode	:	Endpoint
Primary Wave length	:	660 nm
Units	:	%
Flow cell Temp	:	37 °C
Blank	:	Reagent
Calibration type	:	Multi Standard Calibration(Spline)
Reagent volume	:	300 µl (R1) + 100 µl (R2)
Sample volume	:	8 µl
Incubation	:	5 + 5 min.
Low Normal	:	3.8
High Normal	:	5.8
Sensitivity	:	2
Linearity	:	Highest calibrator value
Reaction Slope	:	Increasing

**REFERENCES**

1. Trivelli, L.A., Ranney, H.M., and Lai, H.T., New Eng. J. Med. 284, 353 (1971).
2. Gonen, B., and Rubenstein, A.H., Diabetologia 15, 1 (1978).
3. 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).
4. Bates, H. M., Lab. Mang., Vol 16 (Jan. 1978)
5. ISO 15223-1:2021 Medical devices — Symbols to be used with information to be supplied by the manufacturer — Part 1: General requirements

**Index of Symbols**

 Consult instructions for use	 Catalogue number	 Caution
 <i>In vitro</i> diagnostic medical device	 Batch code	 Non-sterile
 Temperature limit 2-8°C	 Do not re-use	 Use-by date
 Manufacturer	 Date of manufacture	 Keep dry
 Do not use if package is damaged		 Keep away from sunlight



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