

HbA1c Direct Turbidimetry

Only for *in vitro* use in clinical laboratory
Store at 2-8°C

HBA1-200A

Ratio 3:1

HbA1c Reagent



■ Principle of test

This reagent provides a quantitative assay for measuring concentrations of HbA1c in whole blood or red blood cell. It is based on latex agglutination.

HbA1c test samples are absorbed onto the surface of latex particles, which react with Anti-HbA1c (antigen-antibody reaction). The turbidity caused by latex agglutination is measured at 660 nm, and the HbA1c concentration in whole blood or red blood cell is calculated from calibration curve.

■ Clinical significance

Diabetes Mellitus is a chronic disease characterized by a hyperglycemia. The consequences are metabolism disorders of carbohydrates, lipids and proteins. The risk of complications associated with diabetes, including nephropathy, retinopathy and cardiovascular diseases, increases in patients with poor metabolic control. In the diabetic patients, where blood glucose levels are elevated, HbA_{1c} is formed as a consequence of the non-enzymatic glycation of the N-terminus of the β -chain of haemoglobin molecule. The level of HbA1c is proportional to the level of glucose in the blood and has been widely accepted as an indicator of the mean daily blood glucose concentration over the preceding 6-8 weeks. It is therefore, a long-term indicator of diabetic control, whereas, the measurement of blood glucose is only a short-term indicator.

■ General Precautions

- Diagnosis should be made comprehensively on the basis of other relevant test results and clinical symptoms.
- Any change or modification in the procedure described in the package insert may affect the reliability of test results.
- This product contains sodium azide as a preservative. In case reagents accidentally contacted either with eyes, mouse or skin, clean up the contacted parts with plenty of water as first-aid treatment, consult a doctor, if such is required.

■ Kit components

R1; Latex solution
R2; Mouse anti-HbA1c monoclonal antibody, Goat Anti-mouse IgG

■ Procedural Precautions

1. Properties and collection of test samples.

- 1) Collect blood samples with EDTA.
- 2) When measuring on the day of collecting blood samples, centrifuge the blood sample and collect packed red cell as a sample.
- 3) Blood samples should be stored at 2-8 °C and tested within 3 days. Do not freeze blood samples to avoid hemolysis.
- 4) Pick up 15 μ L blood sample using micropipette, and hemolyze with dilution buffer 500 μ L. Then diluted sample is tested.

2. Interference

No interference below concentration.

- 1) Free type bilirubin: 48mg/dl
- 2) Conjugated type bilirubin: 4.8mmol/L
- 3) Triglycerides : 2000mg/dl
- 4) Ascorbic acid: 40mg/dl

3. Handling precautions

- 1) Accurately dispense samples because the dispensing process influences the precision. Change tips each time to avoid errors due to cross-contamination.
- 2) The calibration curve should be prepared for each set of assays since the reaction is affected to some extent by time and temperature.

■ Whole Blood Bench Top Lysis Procedure

- 1) Dispense 500 μ L of Lysis reagent in a sample cup or an Eppendorf microfuge tube.
- 2) Prior to testing, whole blood samples should be mixed by gentle inversion at least 5 times to resuspend settled erythrocytes. **Accuracy of the assay will be affected if whole blood is not mixed prior to testing.** Add 15 μ L of fully resuspended whole blood sample to the lysis buffer in the sample cup or microfuge tube. Mix gently with a suitable pipettor without creating foam and incubate at room temperature (25°C) for 5-10 minutes to completely lyse the red blood cells. Complete lysis is observed when the mixture becomes a clear dark red solution without any particulate matter. Incubate the samples longer as needed to ensure complete hemolysate preparation. The lysate, thus prepared, is ready for use in the Direct Turbidimetric HbA1c assay steps and is stable up to 7 days at 2-8 °C.
- 3) The calibrators and controls should be treated exactly as patient samples and used per instructions on labeling.
- 4) Direct Turbidimetric HbA1c assay reagents are comprised of redox balanced components.

PROCEDURE FOR ANALYZERS

1. Assay conditions:
Wavelength: 660 nm
Temperature37°C

	Blank	Calibrator	Sample
R1 (μ L)	180	180	180
H ₂ O/Cal/Lysate (μ L)	5	5	5

3. Mix and incubate 5 minutes.
3. Read the absorbance (A1) of the calibrator and the samples, at 660 nm against the blank.

4. Add:

	Blank	Calibrator	Sample
R2 (μ L)	60	60	60

5. Mix and incubate 8 minutes.
6. Read the absorbance (A2) of the calibrator and the samples, at 660 nm against the blank.

CALIBRATION

The Direct Turbidimetric HbA1c assay requires weekly (168 hours) calibration. Place calibration series on the analyzer in the order of lowest to highest. Enter calibrator lot specific values provided on the specification sheet.

Direct Turbidimetric HbA1c calibrator sets are intended for use with Hemoglobin A1c Turbidimetric assay reagents. All calibrator vials are stable until their expiration date when stored at 2-8°C.

HbA1c calibrator set is in lyophilized form.

HbA1c calibrator set for the auto analyzers on-Board Lysis Application includes four levels of calibrator material. Levels 1-4 are in lyophilized form. Reconstitute lyophilized contents per instructions on labeling and mix gently. Let the vials equilibrate at room temperature for 30 minutes before use. Reconstituted calibrators are stable for 14 days when capped tightly and stored at 2-8°C.

■ Reference Normal Values

4.5% – 6.5% (NGSP/DCCT)

27 – 46 mmol/mol (IFCC)

Each laboratory should establish its own reference range to reflect the age, sex, diet and geographical location of the population.

■ Performances

Linearity: HbA1c assay has a linear range from 2% - 16.0%.

Precision Studies:

Repeatability (Within Run)(intra-assay): The within run precision was established by assaying two blood samples following NCCLS protocol EP5 on a Hitachi 917.

Level	Mean	Std.Dev.	CV%
Low 5.46	0.074	1.45	
High 10.1	0.169	1.73	

Reproducibility (Run to Run)(inter-assay): The between day precision was established by assaying two blood samples following NCCLS protocol EP5 on a Hitachi 917.

Level	Mean	Std.Dev.	CV%
Low 5.46	0.156	2.81	
High 10.1	0.268	2.72	

Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

Stable glycosylated hemoglobin serves as a substrate for Turbidimetric reaction used in our Direct Turbidimetric HbA1c assay.

■ Precautions for Use or Handling

- (1) This product is intended for *in vitro* diagnostic use only do not use in vivo.
 - (2) Latex and sample diluent in this kit contain sodium azide as preservative.
 - (3) Avoid freezing or drying the latex because it may cause nonspecific agglutination.
 - (1) Strictly follow the storage conditions for this kit.
 - (2) Do not use the reagents after the expiration date.
 - (3) The reagents in this kit are arranged to ensure accurate reaction.
- Do not interchange reagents between different lots numbers.
(4) R1 and R2 are over-turning before using.