PATHOZYME DIAGNOSTICS

# HEMOGLOBIN A1c (HbA1c)

## (TURBIDIMETRY METHOD)

**CAT NO.: HBA**

**Reagent kit for quantitative estimation of HbA1c (Total Hemoglobin Ratio %) in Human Blood.**

### DIAGNOSTIC SIGNIFICANCE:

The clinical significance of glycosylated hemoglobin: **1)** It reflects the average blood glucose level of the patient during a period of 4 to 8 weeks before the blood test, not a single blood glucose. Urine sugar reflects the blood glucose level at the time of blood draw. **2)** Glycated hemoglobin can not only be used as an indicator for monitoring the condition of diabetes, but also as an indicator for early diagnosis of mild, type II, and "hidden" diabetes. But it is not a sensitive indicator for diagnosing diabetes and cannot replace the current glucose tolerance test. **3)** Glycated hemoglobin is used as a monitoring indicator to understand the patient's recent blood sugar status, and to evaluate the occurrence and development of chronic complications of diabetes (diabetic nephropathy, arteriosclerosis, cataracts, etc.). **4)** Prevention of giant fetuses, malformed fetuses, stillbirths, and supervision of the occurrence and development of acute and chronic complications in pregnant women with diabetes is of great significance. **5)** for rescuers whose cause is not clear or are in a coma or undergoing glucose infusion (the blood sugar is of course increased), urgent examination of glycosylated hemoglobin has the value of differential diagnosis. **6)** for diabetic patients with particularly increased glycosylated hemoglobin, we should be alert to the occurrence of acute complications such as ketoacidosis.

### 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 measured absorbance is proportional to the HbA1c absorbed on to the surface of latex partials, which in turn is proportional to the percentage of HbA1c in the sample.

### SPECIMEN COLLECTION:

Whole Blood, recommended using EDTA potassium salt or heparin ammonia, heparin lithium as anticoagulants.

Whole blood stability : 1 weeks at 2-8°C
Lysate stability : 10 hours at 15-25°C
Lysate stability : 10 days at 2-8°C

### KIT PRESENTATION:

| Pack Size | 1 X 20 ml | 1 X 40 ml | 1 X 60 ml |
|---|---|---|---|
| R1 - HbA1c (Latex Reagent) | 1 X 15 ml | 1 X 30 ml | 1 X 45 ml |
| R2 - HbA1c (Buffer Reagent) | 1 X 05 ml | 1 X 10 ml | 1 X 15 ml |
| R3 - HbA1c (Lyse Reagent) | 1 X 50 ml | 2 X 50 ml | 3 X 50 ml |
| HbA1c Calibrator | 4 Vial Set | 4 Vial Set | 4 Vial Set |

### WORKING REAGENT PREPARATION:

R1–HbA1c (Latex Reagent), R2–HbA1c (Buffer Reagent) and R3–HbA1c (Lyse Reagent) are Ready To Use.

### REAGENT STORAGE AND STABILITY:

All reagents are stable at 2-8°C until the expiry date stated on the label.

Once opened, the reagents are stable for 30 days when refrigerated on the analyzer or refrigerator.

### PREPARATION OF CALIBRATOR:

***Reconstitute the calibrators with Glass Distilled water or Deionized water as per quantity mentioned on calibrator vial.*** After reconstitution of calibrator, let it stand for 15 minutes at RT. Then swirl the vial/bottle gently. Properly mix the and check for clarity. Do not shake it vigorously.

### Stability of Reconstituted HbA1c Calibrators:

Reconstituted HbA1c calibrator is stable for 7 days at 2-8°C.Keep the caps tightly close during storage.

### SPECIMEN PRETREATMENT:

Thoroughly mix anticoagulated whole blood. Take 500 µl of Lyse Reagent (R3) and add 10 µl of whole blood for hemolysis. This Lysed sample is referred as **Lysate.** Pipette out 20 µl Lysate (Lysed sample) to run test.

### ASSAY PARAMETERS FOR FIX TIME KINETIC:

| | | | |
|---|---|---|---|
| Reaction | : Fix Time | Sample Volume | : 20 µl |
| Wavelength | : 630 nm | R1 + R2 Volume | : 750 µl + 250 µl |
| Flow Cell Temp. | : 37°C | Calibrator Conc. | : As On Vial |
| Initial Delay | : 30 Sec | Reaction Slope | : Increasing |
| Interval Time | : 270 Sec | Zero Setting | : Dist. Water |
| Read Time | : 270 Sec | Linearity | : 16 % |
| No. of Reading | : 01 | Light Parth | : 1 cm |

### PROCEDURE FOR FIX TIME KINETIC:

| Addition Sequence | Calibrator | Test |
|---|---|---|
| R1 – HbA1c (Latex Reagent) | 750 µl | 750 µl |
| HbA1c Calibrator | 20 µl | -- |
| Sample (Test) | -- | 20 µl |
| Mix well and incubate for 05 minutes at 37°C | | |
| R2 – HbA1c (Buffer Reagent) | 250 µl | 250 µl |

Mix immediately and read **first** absorbance of test exactly at 30 seconds and then, **second,** at an interval of 270 seconds at 630 nm. Determine the change in absorbance (ΔAbs) and calculate the test results.

### ASSAY PARAMETERS FOR END POINT:

| | | | |
|---|---|---|---|
| Reaction | : End Point | Sample Volume | : 20 µl |
| Wavelength | : 630 nm | R1 + R2 Volume | : 750 µl + 250 µl |
| Incubation Temp. | : 37°C | Calibrator Conc. | : As On Vial |
| Incubation Time | : 5 + 5 mins | Reaction Slope | : Increasing |
| Zero Setting | : Dist. Water | Linearity | : 16 % |

### PROCEDURE FOR END POINT:

| Addition Sequence | Calibrator | Test |
|---|---|---|
| R1 – HbA1c (Latex Reagent) | 750 µl | 750 µl |
| HbA1c Calibrator | 20 µl | -- |
| Sample (Test) | -- | 20 µl |
| Mix well and incubate for 05 minutes at 37°C | | |
| R2 – HbA1c (Buffer Reagent) | 250 µl | 250 µl |

Mix well and incubate at 37°C for 5 minutes. Read absorbance of Calibrators and Test after 5 minutes at 630 nm.

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Expiry Date | IVD In-Vitro Diagnostics Use | Storage | Mfg. Date | LOT Batch Number | REF Catalogue Number | See Package Insert

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**MULTI POINT CALIBRATION:**
It is recommended to use the HbA1c calibrators provided with kit for multi-point calibration. Calibration adopts non-linear calibration mode. According to the requirements of the calibration procedure in the operation manual of biochemistry analyzer. *Use the calibrators in increasing order of concentration i.e. lower to higher, during multi point calibration.*

**REQUIREMENTS FOR CALIBRATION AND FREQUENCY:**
It is recommended to calibrate at least once every week. When the following situations occur, it is recommended to re-calibrate: change the reagent batch number, the indoor quality control runs out of control, the biochemistry analyzer carries out major maintenance or replaces the main parts such as light source or cuvette.

**CALCULATION OF RESULT:**
According to the project-specific calibration mode, after the instrument automatically generates a calibration curve, the content of the test substance is calculated from the change in absorbance.

**NORMAL RANGE:**
4% - 6%
Laboratories are suggested to establish its own reference values according to age, sex, diet and region.

**MEASURING RANGE:**
2% - 16%

**LINEARITY:**
This method is linear up to **16 %**.

**QUALITY CONTROL:**
It is recommended to use HbA1c controls. If the results deviate from the scope, please find out the reason by following steps:
1. Check the parameter setting and light source.
2. Check the cleanliness of the cuvette and sampling needle.
3. Check whether water is contaminated or not. Bacterial growth can lead to incorrect results.
4. Check the reaction temperature.
5. Check the validity of the kit.

**INTERFERENCES:**
The effect of Bilirubin ≤ 50mg/dl, Intralipid < 700 mg/dl, Ascorbic Acid ≤ 50mg/dl is less than 10%.

**SAFETY PRECAUTIONS AND WARNINGS:**
1. The reagent contains preservatives. If it enters the eyes, mouth or contact on the skin, please rinse it thoroughly with clean water immediately and go to the hospital if necessary.
2. The reagent contains preservatives, which can react strongly with copper, lead and other metals to form Azide metal. Therefore, please dilute the waste liquid and flush the drain pipe to avoid residual when disposal.
3. Do not mix or exchange reagents with different batches in the process of detection.
4. Opened reagents should be sealed and stored according to the specified method. Expired product should not be used.
5. Please dispose test tubes and other instruments that have touched the test sample according to the relevant medical waste disposal regulations.

**WASTE DISPOSAL**: This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

**REFERENCES:**

1. Goldstein DE, Little RR, Lorenz RA, et al. Tests of glycemia in diabetes. Diabetes Care 1995;18:896- 909.
2. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977-986.
3. American Diabetes Association, "Standards of Medical Care for Patients with Diabetes Mellitus", (Position statement), Diabetes Care, 21 (Suppl.1): S23 S31 (1998).
4. Pantheghini M, John WG on behalf of the IFCC Scientific Division. Implementation of haemoglobin A1c results traceable to the IFCC reference system: the way forward. Clin Chem Lab Med 2007; 45(8):942-4



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Expiry Date

In-Vitro Diagnostics Use


Storage


Mfg. Date



Batch Number



Catalogue Number


See Package Insert


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