# Package Insert

**REF** SC-HB001 | **English**

*A rapid test for semi-quantitative estimation of HbA1c in human whole blood specimen. For laboratory professional in vitro diagnostic use only.*

**【INTENDED USE】**

The HbA1c Rapid Test Cassette (Whole Blood) is a rapid chromatographic immunoassay for the semi-quantitative estimation of human HbA1c in whole blood as an aid in the monitoring of diabetes mellitus.

**【SUMMARY】**

**Glycated hemoglobin** (**hemoglobin A1c**, **$HbA_{1c}$**, **A1C**, or **$Hb_{1c}$**; sometimes also referred to as being **Hb1c** or **HGBA1C**) is a form of hemoglobin that is measured primarily to identify the three-month average plasma glucose concentration. The test is limited to a three-month average because the lifespan of a red blood cell is four months (120 days). However, since red blood cells do not all undergo lysis at the same time, HbA1c is taken as a limited measure of three months. It is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose.

$HbA_{1c}$ is a measure of the beta-N-1-deoxy fructosyl component of hemoglobin.[1] The origin of the naming derives from Hemoglobin type A being separated on cation exchange chromatography. The first fraction to separate, probably considered to be pure Hemoglobin A, was designated $HbA_0$, the following fractions were designated $HbA_{1a}$, $HbA_{1b}$, and $HbA_{1c}$, respective of their order of elution. There have subsequently been many more sub fractions as separation techniques have improved.[2] 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. This serves as an indicator that blood sugar is increasing and that action should be taken.

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. A trial on a group of patients with Type 1 diabetes found that monitoring by caregivers of $HbA_{1c}$ led to changes in diabetes treatment and improvement of metabolic control compared to monitoring only of blood or urine glucose.[3] However, a trial designed specifically to determine whether reducing $HbA_{1c}$ below the normal 6%, using primarily insulin and sulfonylureas (both known to easily drive blood sugar too low), would reduce the rate of cardiovascular events in type 2 diabetes found *higher* mortality—the trial was terminated early.[4] The negative outcomes may well have been a result of the treatment approach, primarily insulin and sulfonylureas, utilized in the "intensive" treatment group instead of LCHF (Low-Carbohydrate High Fat diet), GIP-1 analogues & SGLT-2 inhibitors, none of which have these problems & lower cardiovascular mortality.

**【PRINCIPLE】**

The HbA1c Rapid Test Cassette (Whole Blood) is a semi-quantitative, membrane based immunoassay for the detection of HbA1c in human whole blood. The membrane is pre-coated with anti-hemoglobin antibodies. During testing, the HbA1c in whole blood specimen reacts with anti-HbA1c part of the dye conjugate, which has been impregnated on the conjugate pad. The mixture then migrates upward on the membrane by capillary action, reacts with anti-hemoglobin on the membrane on Test Line region. If the specimen contains HbA1c, a colored line will appear in test line region. The absence of the colored lines in test line region indicates that the specimen does not contain HbA1c, or the concentration of HbA1c is lower than cut-off value. To serve as a procedure control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

**【REAGENTS】**

The test cassette includes anti-HbA1c coated particles and anti-hemoglobin coated on the membrane.

**【PRECAUTIONS】**

- For laboratory professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used test should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.

**【STORAGE AND STABILITY】**

The kit can be stored at room temperature or refrigerated (2-30°C). The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. **DO NOT FREEZE**. Do not use beyond the expiration date.

**【SPECIMEN COLLECTION AND PREPARATION】**

**Preparation**

Before performing the test, please make sure that all components are brought to room temperature (15-30°C).

1. Take a tube with buffer solution out of the kit. Document patients' name or ID on it. Open the screw cap.

**Blood Sample Taking**

2. Collect the specimen according to standard procedures.
   - Do not leave specimens at room temperature for prolonged periods. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be used within 3 days of collection. Do not freeze whole blood specimens.
   - EDTA K2, Heparin sodium, Citrate sodium and Oxalate potassium can be used as anti-coagulants.

**Sample Dilution / Sample Stability**

3. Administer the blood-filled end-to-end capillary into the plastic tube with dilution buffer. Alternatively, the **10 µL** of specimen can be added directly with the micro pipette into the buffer.
4. Close the tube and shake the sample by hand forcefully for approximately **10 seconds** so sample and dilution buffer mix well.
5. Let the diluted sample rest for approximately 1 minute.
6. The diluted sample can then be used immediately or stored for up to 24 hours at 2-8°C.

**【MATERIALS】**

**Materials Provided**

- Test Cassettes
- Package Insert
- Plastic Tubes with Buffer
- Droppers
- Capillaries
- Color Card

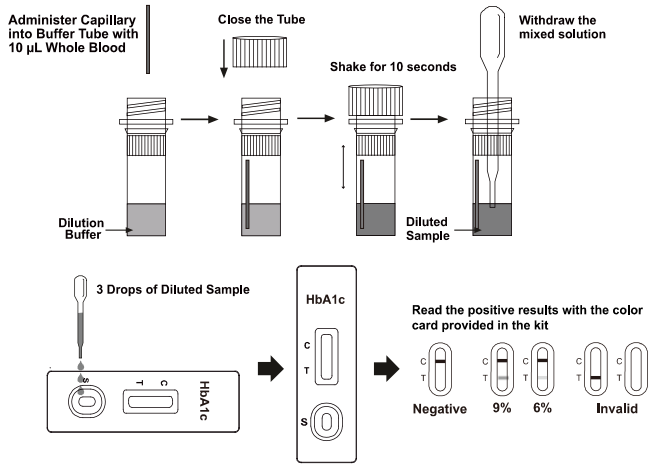
**Materials Required but Not Provided**

- Timer
- Specimen Collection Containers
- Lancets (For fingerstick whole blood use only)

**【DIRECTIONS FOR USE】**

**Bring tests, specimens and buffer to room temperature (15-30°C) before use.**

1. Remove the test cassette from its sealed pouch, and place it on a clean, level surface. For best results, the assay should be performed within one hour.
2. Open the tube with the diluted sample. Transfer **3 drops (approx. 120 µL)** of diluted sample to sample well. Start the timer.
3. Wait for the colored lines to appear. **The result should be read at 10 minutes.** Do not interpret the results at 20 minutes.




**【INTERPRETATION OF RESULTS】**

(Please refer to the illustration above)

**POSITIVE:* Two colored lines appear**. One colored line should be in the control line region (C) and another colored line should be in the test line region (T).

***NOTE:** The intensity of the color in the test line region (T) will vary depending on the concentration of HbA1c present in the specimen. **Read the positive results with help of the color card provided in the kit as your reference to confirm the concentration of HbA1c in the whole blood specimen.**

**NEGATIVE: One colored line appears in the control line region (C).** No colored line appears in the test line region (T), or the test line is weaker than the reference line representing 6% on the color card**.** This indicates HbA1c to be at healthy level meaning excellent control.

**INVALID: Control line fails to appear.** Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

**【QUALITY CONTROL】**

Internal procedural controls are included in the test. Control line appearing in the control region is considered an internal procedural control, confirming sufficient specimen volume and correct procedural technique.

External controls are not supplied with this kit. It is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

**【LIMITATIONS】**

1. The HbA1c Rapid Test Cassette (Whole Blood) is for professional *in vitro* diagnostic use, and should only be used for the semi-quantitative estimation of HbA1c.
2. The HbA1c Rapid Test Cassette (Whole Blood) will only provide semi-quantitative estimation of HbA1c in the specimen to be <6%, 6%-9% or >9% and should not be used as the sole criterion for evaluating Diabetes. Laboratories can have their separate reference values for HbA1c to be under control.
3. Like with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
4. This HbA1c Rapid Test Cassette (Whole blood) is designed to work with hematocrit level between 25% and 65%. Performance of this test kit at a different hematocrit level can lead to erroneous results.

**【EXPECTED VALUES】**

The expected HbA1c values in a healthy individual is <6%. Some studies also put this value at 5.9%.

**【PERFORMANCE CHARACTERISTICS】**

**Detection Limitation**

The HbA1c Rapid Test Cassette (Whole Blood) can detect HbA1c as low as 6%.

**Sensitivity and Specificity**

The HbA1c Rapid Test Cassette (Whole Blood) was compared with commercial HbA1c ECLIA kit; the results indicate that HbA1c Rapid Test Cassette (Whole Blood) has a high sensitivity and specificity.

| **Method** | | **ECLIA** | | **Total Results** |
|---|---|---|---|---|
| **HbA1c Rapid Test Cassette (Whole Blood)** | **Results** | Positive | Negative | |
| | Positive | 75 | 2 | 77 |
| | Negative | 0 | 148 | 148 |
| **Total Results** | | 75 | 150 | 225 |

Relative Sensitivity: ＞99.9% (95%CI*: 96.1%-100%) *Confidence Interval

Relative Specificity: 98.7% (95%CI*: 95.3%-99.8%)

Overall Accuracy: 99.1% (95%CI*: 96.8%-99.9%)

**Precision**

**Intra-Assay**

Within-run precision has been determined by using 3 replicates of the following specimens: negative, 6% HbA1c and 9% HbA1c. The negative, 6% HbA1c and 9%HbA1c values were correctly identified >99% of the time.

**Inter-Assay**

Between-run precision has been determined by 3 independent assays on the same specimens: negative, 6% HbA1c and 9% HbA1c positive specimen. Three different lots of the HbA1c Rapid Test Cassette (Whole Blood) have been tested over a 3-days period using negative, 6% HbA1c and 9% HbA1c positive specimens. The specimens were correctly identified >99% of the time.

**Cross-reactivity**

The HbA1c Rapid Test Cassette (Whole Blood) has been tested for HBsAg, anti-HIV, anti-HCV, anti-RF, anti-Spyhilis, anti-*H.pylori*, anti-Toxo IgG, anti-Rubella IgG, anti-CMV IgG positive specimens. The results showed no cross-reactivity.

**Interfering Substances**

The following compounds have also been tested using the HbA1c Rapid Test Cassette (Whole Blood) and no interference was observed.

| | | |
|---|---|---|
| Acetaminophen: 20 mg/dL | Caffeine: 20 mg/dL | Creatin: 200 mg/dL |
| Acetylsalicylic Acid: 20 mg/dL | Gentisic Acid: 20 mg/dL | Albumin: 2000 mg/dL |
| Ascorbic Acid: 2 g/dL | Hemoglobin: 1000 mg/dL | Oxalic acid: 600 mg/dL |
| Bilirubin: 1000 mg/dL | | |

**【BIBLIOGRAPHY】**


1. Miedema K (2005). "Standardization of HbA1c and Optimal Range of Monitoring". Scandinavian Journal of Clinical and Laboratory Investigation. 240:61–72.
2. Peterson KP, Pavlovich JG, Goldstein D, Little R, England J, Peterson CM (1998)."What is hemoglobin A1c? An analysis of glycated hemoglobins by electrospray ionization mass spectrometry". Clinical Chemistry. 44 (9): 1951–58.
3. Larsen ML, Hørder M, Mogensen EF (1990). "Effect of long-term monitoring of glycosylated haemoglobin levels in insulin-dependent diabetes mellitus". N. Engl. J. Med. 323 (15): 1021–25.
4. Gerstein HC, Miller ME, Byington RP, et al. (2008). "Effects of Intensive Glucose Lowering in Type 2 Diabetes". New England Journal of Medicine. 358(24): 2545–59.


**Index of Symbols**

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|---|---|---|---|---|---|
|  | Caution |  | Tests per kit |  | Consult Instructions For Use |
|  | Manufacturer |  | Use by |  | Do not reuse |
|  | Store between 2-30°C | LOT | Lot Number | REF | Catalog # |
|  | Do not use if package is damaged | | | | |


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