# Hemoglobin A1c (HbA1C) Specification

## (Enzymatic Assay Method)

**【Product name】**

Generic Name :Hemoglobin A1c Kit (Enzymatic Assay Method)

Abbreviated name: HbA1c

English name：Glycohemoglobin A1c (HbA1c) Test Kit

**【Intended use】**

In vitro test for the quantitative determination of HbA1c concentration in human whole blood on photometric systems.

HbA1c is a product of hemoglobin (Hb) that produces a slow and continuous non-enzymatic glycation reaction under the action of high blood glucose. Glucose modifies hemoglobin specifically in its n-terminal valine residue to form glycated hemoglobin. Under normal physiological conditions, the production of non-enzymatic glycosylation reaction products is proportional to the concentration of the reactants. Since the hemoglobin concentration keeps relatively stable, the glycosylation level is mainly determined by the glucose concentration and also depends on the duration of contact between hemoglobin and glucose. As a result, HbA1c reflects the average blood glucose level during the preceding 2 to 3 months.

**【Test principle】**

Simultaneously the fructosyl dipeptides are generated from the N-terminus amino groups of the beta-chain of HbA1c by the reaction of protease. In the first reaction, the concentration of hemoglobin is measured at absorbance in 500nm. In the second reaction, the reaction of Fructosyl peptide oxidase(FPOX) with fructosyl dipeptides, generated hydroperoxide allows 10-(carboxymethylaminocarbonyl)-3,7-bis(dimethylamino) phenothiazine sodium salt to develop a color in the presence of peroxidase. The absorbance in 660nm is measured to calculate HbA1c concentration. The combined assay results for hemoglobin and HbA1c are used by the system to calculate and express HbA1c%.

**【Main components】**

| | Composite | Concentration |
|---|---|---|
| HbA1c | **Reagent 1（R1）：** | |
| | Good's buffer solution | 100mmol/L |
| | Protease（PRK） | 500ku/L |
| | DA-67 | 10mmol/L |
| | **Reagent 2（R2）：** | |
| | FPOX | 50KU/L |
| | Good's buffer solution | 100mmol/L |
| | **Reagent 3（Sample treatment solution）** | |
| | Good's buffer solution | 100mmol/L |

**【Storage and shelf life】**

Its shelf life is 12 months, when stored unopened at 2-8℃ and protected from light. Once opened, the reagents are stable for one month when stored unopened at 2-8℃ and protected from light. Contamination of the reagents must be avoided. Do not freeze the reagents.

**【Samples requirement】**

1. No hemolytic anticoagulation whole blood, use EDTA anticoagulation.
2. After centrifuge of whole blood or samples (2000rpm, 5min), take 25μL whole blood or red blood cell after centrifuge, add to 500 μ L samples treatment solution, mix and assay it as treatment samples in Automatic biochemical analyzer.

**【Applicable instrument】**

Hitachi 7180/7170/7060/7600 Automatic biochemical analyzer. Abbott 16000, OLYMPUS AU640 Automatic biochemical analyzer.

**【Assay Method】**

1.Reagent preparation

R1：can be used directly after opening;

R2：can be used directly after opening.

Assay condition：

⑴ Non-twin test

① Hb Assay

Assay Procedure：

| | | | |
|---|---|---|---|
| Main wave length | 500nm | Sub wave length | / |
| Temperature | 37℃ | Analysis type | 2-End point |
| Samples treatment (calibration) | | 12μL | |
| R1 | | 180μL | |
| Mix and incubate for 5 min, at 37℃, and read the absorbance A at 500nm. | | | |

△A=A sample / calibrator -A blank

② HbA1c Assay

Assay Procedure：

△A=[（A2-A1）calibrator/sample]-[（A2-A1）blank]

| | | | |
|---|---|---|---|
| Main wave length | 660nm | Sub wave length | 800nm |
| Temperature | 37℃ | Analysis type | 2-End point |
| Samples treatment（calibration） | | 12μL | |
| R1 | | 180μL | |
| Mix and incubate for 5 min, at 37℃, and read the absorbance A at 500nm, the absorbance A1 at 660nm | | | |
| R2 | | 60μL | |
| Mix thoroughly at 37℃ and read the absorbance A2 at 660nm again 5 min later. | | | |

⑵Twin test

Test method：

Pr-treatment sample 12 μ L → R1 180 μ L, 37℃ 5min → Measure Absorbance Ⅰ → + R2 60 μ L, 37℃ 5min → Measure Absorbance Ⅱ → Calculate concentration

Absorbance Ⅰ： Absorbance difference in 505nm with 800nm and Absorbance difference in 660nm with 800nm.

Absorbance Ⅱ： Absorbance difference in 660nm and 800nm

Note：The instrument with twin test must meet below requirements:

①Different detected items can corresponding to the same reagent position.

②It can measure absorbance in different wavelength under the same reaction cup

3. Calculation method

HbA1c% was directly used to represent the results of HbA1c, which should be calculated according to the concentration of HbA1c and HbA1c. In order to cope with the standardized HbA1c values recommended by different institutions, the inter-project calculation formula by automatic analyzer is required:

a. Standard method from Japanese diabetes association

HbA1c=96.3*HbA1c（μmol/L）/Hb（μmol/L）+1.62

b.Standard calculation method of HbA1c from the USA/Methods of diabetes control and complications（NGSP/DCCT method):

HbA1c=91.5*HbA1c（μmol/L）/Hb（μmol/L）+2.15

c.IFCC recommended method
HbA1c=HbA1c（μmol/L）/Hb（μmol/L）*100

**【Reference intervals】**
≤6.5%
Determination method：Select blood samples from no less than 100 normal people through clinical trials, and test by Automatic biochemical analyzer. The measured values are statistically processed and the reference interval is calculated.
It is suggested that each laboratory establish its own reference range!
【The limitations of the test method】
Bilirubin in the sample≤40mg/dL、carbamyl Hb≤7.5mmol/L, acetylation Hb≤5.0 mmol/L, chylomicron ≤ 200 Turbidity unit, ascorbic acid ≤ 50mg/dL. No obvious interference was observed, and the concentration was calculated as the concentration in the untreated samples.
【Interpretation of test results】
Human error, specimen handling and deviation of analytical instrument can affect the measurement results. When individual samples deviate too far from the expected value, they need to be measured again.

【Product performance index】
1.Under the condition of 37℃, 660nm wavelength, 1cm optical path, when using purified water as sample to be added to reagent to test, the change of reagent blank absorbance should not exceed 0.10.
2. Accuracy：Repeat ability CV is no more than 5%. The relative range R between batches shall not be exceed 10%.
3. The degree of accuracy: Test traceable standards;Within the range of 3%-16%, the relative deviation should not exceed ±10%
linearity range:1）When Hb is 90~300 μ mol/L, the saccharification percentage is within the range of 3% to 16%, and the correlation coefficient r should not be less than 0.990.The relative deviation between the measured concentration and the estimated value should not exceed ±10%.
5. sensitivity for analysis: When HBA1C is tested with this reagent, the absorbance variation ΔA caused by 10 μ mol/L concentration is no less than 0.01.
6. Stability: It could be used before shelf life when all dosage forms should be stored at 2~8℃ under the condition of avoiding light strictly.
【Attention】Please don't mix the reagent with different batch number or use it, please calibrate it again when reagent batch number change. replace the batch number reagent, please re-calibration!