

### General Precautions

1. This product is for *in vitro* diagnostic use only, and must not be used for any other purposes.
2. Clinicians should make a comprehensive clinical decision based on assay results in conjunction with clinical symptoms and other examination results.
3. This product should be used only as directed. Reliability of values cannot be guaranteed if this product is used for purposes or tested by methods other than those stated.
4. If the reagents come in contact with the eyes or mouth, rinse thoroughly with water as first aid, and seek for medical treatment if necessary.
5. Read the user's manual of your automatic analyzer prior to using this kit. Parameters for different automated analyzers are available upon request.
6. Perform a quality control test prior to any assay to ensure accuracy.

### Description (Kit Components)

Component	Ingredients
HbA1c Reagent 1	10- (carboxymethyl aminocarbonyl) -3,7-bis (dimethylamino) phenothiazine sodium salt
HbA1c Reagent 2	Peroxidase Fructosyl peptide oxidase

### Intended Use

#### For the measurement of the glycated hemoglobin (HbA1c) in human whole blood

HbA1c is a glycosylated product of hemoglobin A0 (HbA1c), the predominant form of hemoglobin in adults. Measurement of the percentage of HbA1c reflects the mean blood glucose concentration over the preceding one to two months, and is therefore considered to be an important diagnostic marker for monitoring blood glucose levels. 1)

### Assay Principle

#### 1. Assay principle

In the first reaction, protease cleaves the glycosylated dipeptide from the N-terminal beta-chains of HbA1c, and the concentration of hemoglobin is measured at a given wavelength. In the second reaction, glycosylated dipeptide reacts with fructosyl peptide oxidase (FPOX) and generates hydrogen peroxide. Hydrogen peroxide, in the presence of peroxidase (POD), reacts with 10-(carboxymethyl aminocarbonyl) -3,7-bis (dimethylamino) phenothiazine sodium salt (coloring agent) to develop color.

The change in absorbance is measured to determine the concentration of HbA1c. The combined assay results for hemoglobin and

HbA1c are used by the system to calculate HbA1c (%)

HbA1c:



#### 2. Features

- 1) Enzymatic method specifically measures N-terminal fructosyl dipeptides of the beta-chain of HbA1c.
- 2) Ready-to-use liquid reagents
- 3) Reaction cuvettes will remain clean after the reaction.

- 4) Compatible with various types of automated analyzers.
- 5) Assay results will not be affected by unstable forms of HbA1c and other types of modified hemoglobin.

### Procedural Precautions

#### 1. Sample Collection and Storage

##### 1) Samples

- i) Whole blood samples (EDTA, NaF-EDTA, heparin) can be used.
- ii) Use **Pretreatment Solution (Reagent 3) HbA1c** for sample pretreatment.
- iii) Whole blood that is significantly hemolyzed must not be used.

##### 2) Sample Storage

- i) Blood samples are stable for 7 days if stored refrigerated.
- ii) Pretreated samples are stable for up to 8 hours at room temperature, and up to 24 hours if stored refrigerated. Pretreated samples stored for more than 2 hours should be mixed well. Samples must be brought to room temperature (15-30°C) prior to assay.

#### 2. Interfering Substances

- 1) Assay results will not be affected by free bilirubin (up to 50 mg/dL), conjugated bilirubin up to 50 mg/dL, ascorbic acid (up to 50 mg/dL) and Intralipos (parenteral fat emulsions) level of up to 2%, formazin turbidity (up to 3000 units).
- 2) Assay results will not be affected by hemoglobin modified by acetaldehyde, acetylsalicylic acid, sodium cyanide (up to 50mg/dL).
- 3) Assay results will not be affected by unstable HbA1c formed by glucose in concentrations of up to 1000mg/dL.
- 4) Handle samples from patients receiving intravenous amino acid solutions with extreme caution. Avoid using samples from patients receiving amino acid transfusion, in which the amino acids and glucose are mixed in advance, as measurements obtained from such samples may result in abnormally high values.

#### 3. Other Precautions

- 1) Use **Calibrator for Audit Diagnostics HbA1c** as the calibration material.
- 2) If the hemoglobin concentration in the pretreated sample exceeds 310µmol/L, pretreat the sample twice the usual amount with **Pretreatment Solution** and retest. If the hemoglobin concentration is below 90µmol/L pretreat the sample with half the usual amount of the pretreatment solution and retest.

### Assay Procedure

#### 1. Preparation

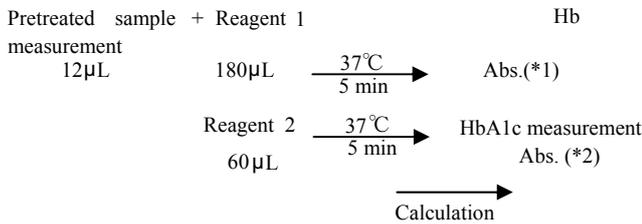
Reagent 1 - HbA1c Reagent 1: Ready-to-use  
Reagent 2 - HbA1c Reagent 2: Ready-to-use

#### 2. Sample Preparation (Manual method)

- 1) Add 500µL of **Pretreatment Solution** to the test tube.
- 2) Centrifuge the blood sample for 5 minutes at 800 g. Add a 25 µL aliquot from the blood cells in the lower layer to the pretreatment solution and mix well.

### 3. Assay Method

This product is compatible with various types of automatic analyzers. Below is a general example of the assay procedure.



\*1 Difference in absorbance at 480 nm and 800 nm for Hb measurement and at 660 nm and 800 nm for HbA1c measurement

\*2 Difference in absorbance at 660 nm and 800 nm for HbA1c measurement

To convert the measured values to NGSP values(%) the following equation is applied:

$$\text{HbA1c (NGSP\%)} = 98.2 \times$$

$$\text{HbA1c}(\mu\text{mol/L})/\text{Hb}(\mu\text{mol/L}) + 1.97$$

Calibration material: **Calibrator for HbA1c**

### Assessment of Results

- Reference range<sup>1)3)</sup>:  
4.6-6.2% (NGSP) 4.3-5.8% (JDS)
- There may be reactions with non-target substances or interfering reactions. If measurement results appear unreliable, repeat the measurement (if necessary, after dilution) or try another analytical method.

### Performance

#### 1. Sensitivity

Absorbance of HbA1c per 10 μmol/L: 0.050-0.100

Absorbance of Hb per 100 μmol/L: 0.10-0.3

#### 2. Accuracy

Within 90-110% of the expected assay values

#### 3. Within-run Reproducibility

Coefficient of variation ≤5% (within-run)

(Test methods used for 1.-3. are in-house methods.)

#### 4. Measurement Range

(on Hitachi 7170 automated analyzer)

HbA1c: 3.3-16.6 % (NGSP)

3-16% (JDS)

(Hb concentration : 90-310 μmol/L)

#### 5. Correlation (JDS values)

N=137 r=0.991 y=0.98x+0.04

Reference method: HPLC method

#### 6. Standard Calibration Material

Certified Reference Material for Measurement of HbA1c by IFCC method (JCCRM411-2 (JDSL0t4))

### Precautions and Warnings

#### 1. Handling Precautions

- All samples used in the test should be handled as if potentially infectious for HIV, HBV and HCV. To prevent infection, use disposable gloves and avoid mouth pipetting during the test.
- HbA1c Reagent 1 contains sodium azide as well as Proclin 300 as preservatives, which may irritate the skin. Therefore, if the reagent comes in contact with the skin or clothes, rinse immediately with a large amount of water, and consult a doctor if skin irritation develops.

#### 2. Precautions for Use

- This product should be stored as directed. Avoid freezing. Freezing can cause deterioration of the reagents, which can produce inaccurate results.

- The bottle of HbA1c reagent 1 has been designed to prevent deterioration from sunlight. Do not transfer the reagent to other bottles.
- Keep the cover of the automated analyzer closed during calibration and measurement. If conditions differ during calibration and measurement, inaccurate measurements may result.
- Do not use expired reagents. Reliable assay values cannot be obtained if expired reagents are used.
- Do not replenish the reagents.
- Do not perform the assay under direct sunlight.

### 3. Precautions for Disposal

- Before disposal, used samples and their containers must be soaked in sodium hypochlorite solutions at a concentration greater than 0.1% for more than an hour or autoclaved at 121°C for 20 minutes.
- To prevent infections from spilled samples or sample-containing solutions, wipe the spilled area thoroughly with solutions such as sodium hypochlorite solutions at a concentration greater than 0.1%.
- The reagents and samples should be disposed as medical waste or industrial waste in accordance with waste disposal regulations.
- The reagents should be disposed in accordance with water pollution control regulations or related regulations.
- Reagent 1 contains sodium azide, which can react with lead or copper pipes to produce highly explosive metal azide. Therefore, thereagent should be flushed out with copious amounts of water when discarding.

### 4. Other Precautions

Do not use the containers for other purposes.

### Storage and Shelf life

Description		Storage
<b>Audit Diagnostics HbA1c</b>	Reagent 1	2-10°C, Shield from light
	Reagent 2	2-10°C

(Expiration date is printed on the outside of the package.)

### Package Contents

Description		Configuration
<b>Audit Diagnostics HbA1c</b>	Reagent 1	30 mL x 2
	Reagent 2	10 mL x 2

### References

- Japan Diabetes Society: A guideline for medical treatment of diabetes 2010, p. 9, Bunkodo (2010)
- Japan Society of Clinical Chemistry Committee on the Standardization of Diabetes-Related Laboratory Testing: Guideline for the use of IFCC units for HbA1c Measurements (ver.2.0:2008-10-06) supplement, Clinical Chemistry, 38, 440 - 445 (2009).
- Japan Diabetes Society Homepage "International Standardization of HbA1c Measurements" Reference 1.