

This package insert must be read carefully prior to use.

Glycohemoglobin A1c assay kit
(Classification No.: 30168000)

NORUDIA N HbA1c

General Precautions

1. This product is for in vitro diagnostic use, 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 in this package insert. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.
4. If the reagent accidentally comes in contact with eyes and/or mouth, rinse immediately with ample water as first aid, and consult the doctor if required.
5. Carefully read the operating instructions for each type of automated analyzers prior to using this product. Parameters for each type of analyzers are available, and can be requested from SEKISUI MEDICAL CO., LTD. if required.
6. Perform a quality control test prior to assay to ensure accuracy.

Description (Kit Components)

Component: Ingredients

HbA1c Reagent 1: 10-(Carboxymethyl amino carbonyl)-3,7-bis (dimethylamino) phenothiadine sodium

HbA1c Reagent 2: Peroxidase
Fructosyl peptide oxidase

Intended Use

Measurement of hemoglobin A1c (HbA1c) in whole blood

HbA1c is a glycation product of HbA0 accounting for most of hemoglobin (Hb). The HbA1c concentration reflects the mean blood glucose level over a period of 1–2 months before testing, and it is used as an index of glycemic control in patients with diabetes.¹⁾

Assay Principle

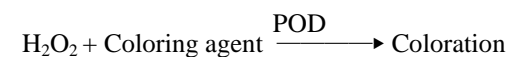
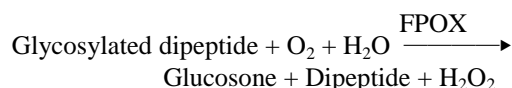
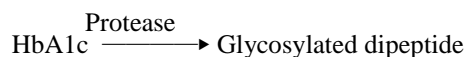
1. Assay Principle

In the first reaction, glycosylated dipeptide derived from the N-terminal of the β-chain of HbA1c is cut out by a protease, and the Hb concentration is subsequently determined from the absorbance at a specified wavelength. In the second reaction, hydrogen peroxide is produced by the action of fructosyl peptide oxidase (FPOX) on glycosylated dipeptide, and then causes color development by 10-(carboxymethyl aminocarbonyl)-3,7-bis (dimethylamino) phenothiazine sodium (coloring agent) in the presence of peroxidase (POD). The HbA1c

concentration is determined by measuring the absorbance of this complex.

The percentage of total Hb existing as HbA1c is calculated from the concentrations of HbA1c and Hb.

HbA1c:



2. Features

- 1) This product is a reagent that specifically measures HbA1c-derived glycosylated dipeptide by the enzymatic method.
- 2) Liquid reagents, ready-to-use.
- 3) Reaction cells are hardly contaminated.
- 4) Applicable to various automated analyzers.
- 5) Analytical results are not affected by unstable HbA1c or modified Hb (such as carbamylated or acetylated Hb).

Procedural Precautions *

1. Properties of Samples and Sampling Methods

1) Samples

- Whole blood (EDTA, NaF-EDTA or heparin) may be used.
- For pretreatment of samples, use Pretreatment Solution for NORUDIA N HbA_{1c}, which is sold separately (manufactured by SEKISUI MEDICAL CO., LTD).
- Do not use highly hemolyzed whole blood.

2) Storage of samples

- Samples are stable for 7 days in refrigeration.
- The stability of samples treated by pretreatment solution for NORUDIA N HbA_{1c} (pretreated samples) is as follows.
Room temperature: 8 hours
Under refrigeration: 24 hours
Stored samples should be brought to room temperature (15–30°C) before use. After storage for 2 hours or longer, thoroughly stir pretreated samples before use.

2. Interfering substances

- 1) Assay results are not affected by free bilirubin (up to 50 mg/dL), conjugated bilirubin (up to 50 mg/dL), formazin turbidity (up to 3000 FTU), or ascorbic acid (up to 50 mg/dL).
- 2) Analytical results are not affected by modified Hb (acetaldehyde-modified, acetylated, or carbamylated) obtained by adding acetaldehyde, acetylsalicylic acid, or sodium cyanate, respectively, to the sample at a concentration of 50 mg/dL.
- 3) Analytical results are not affected by unstable

HbA1c obtained by adding glucose to the sample at a concentration of 1000 mg/dL.

- 4) **Pay attention to measurement of samples collected from patients dosed with amino acid. In particular, do not use samples containing a mixture of glucose and amino acids, or falsely high values may be obtained.**

3. Others

- 1) Always use HbA1c Calibrator for NORUDIA N HbA1c for calibration.
- 2) Precautions for assay range
If the Hb concentration of the treated sample is greater than 310 µmol/L, increase the volume of pretreatment solution to twice the normal volume (if the Hb concentration is 90 µmol/L or lower, decrease the pretreatment solution volume to half of normal), and perform re-measurement.

Dosage/Administration (Assay Procedure) *

1. Preparation of reagents

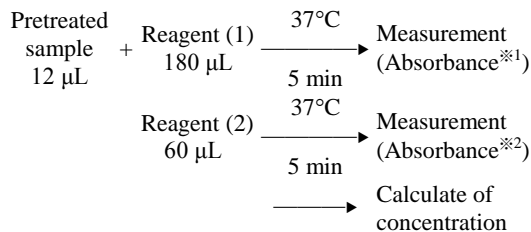
Reagent (1): HbA1c Reagent 1 is ready to use.
Reagent (2): HbA1c Reagent 2 is ready to use.

2. Assay Procedure

- (1) Place 500 µL of Pretreatment Solution for NORUDIA N HbA1c (sold separately) in each test tube in advance.
- (2) After centrifugation of the sample (800 g for 5 minutes), collect 25 µL from the blood cell layer, add it to the pretreatment solution mentioned in Step 1 above, and mix. Measure this mixture with an automated analyzer as the pretreated sample.

3. Assay Procedure

This product is compatible with various types of automated analyzer. An example of the assay procedure is indicated below.



*¹ Absorbance: The difference in absorbance between 480 nm and 800 nm and between 660 nm and 800 nm

*² Absorbance: The difference in absorbance between 660 nm and 800 nm

Inter-item operation expressions for the automated analyzer are required for conversion to NGSP values (%).²⁾

$$\text{HbA1c (NGSP\%)} = 91.5 \times \text{HbA1c } (\mu\text{mol/L}) / \text{Hb } (\mu\text{mol/L}) + 2.15$$

Calibration material: HbA1c calibrator for NORUDIA N HbA1c (Manufacture's assigned value)

Assessment of Assay Results * *

1. Reference standard range¹⁾³⁾

4.6–6.2% (NGSP value)

2. There may be reactions or interfering reactions

with non-target substances. If assay results appear to be unreliable, repeat the measurement (if necessary, after dilution) or try another analytical methods.

3. As there is a risk of assay results being artificially high in a sub-portion of samples with extremely low peroxidase (POD)-like activity, physicians should assess all results comprehensively in conjunction with clinical symptoms and other findings.

Performance *

1. Sensitivity

HbA1c: The absorbance is 0.050–0.100/min per 10 µmol/L of HbA1c.

Hb: The absorbance is 0.10–0.30/min per 100 µmol/L of Hb.

2. **Accuracy:** 90–110% of the expected assay value

4. Within-run Reproducibility:

Coefficient of variation ≤ 5%

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

4. **Measurement Range⁴⁾:** (On Hitachi 7170 automated analyzer)

HbA1c: 3.3–16.6 % (NGSP value)

(If the Hb concentration is 90–310 µmol/L.)

5. **Correlation⁴⁾** (JDS values)

$$N=137 \quad r=0.991 \quad y=0.98x+0.04$$

Control method: HPLC

6. **Standard Material**

Primary Reference Material for Measurement of HbA1c (JCCRM411)

Precautions for Use or Handling *

1. Precautions for Handling (to Ensure Safety)

- 1) All samples used in the test should be handled as a material possibly infected with HIV, HBV, HCV, or other viruses. To prevent infection, use disposable gloves and avoid mouth pipetting during the test.
- 2) Sodium azide is added as an antiseptic agent in the HbA1c Reagent 1. Therefore, if the reagent comes in accidentally contact with eyes, mouth or skin, rinse immediately with ample water as first aid, and consult the doctor if required.

2. Precautions for use

- 1) This product should be stored as directed, without freezing. Freezing can deteriorate the reagents, which can produce inaccurate results. Therefore, avoid using the reagents which have been previously frozen.
- 2) Black vial are used for HbA_{1C} reagent 1 to prevent photo-degradation. Avoid transfer into other vial.
- 3) If the position of the cover of the automated analyzer (open/close) at the time of calibration and measurement differs, accurate results may not be obtained due to the difference in the level of light entering the reaction cell. Close the cover of the automated analyzer during use.
- 4) Do not use expired reagents. Use of such reagents cannot guarantee the reliability of measurement values.
- 5) Do not replenish the reagents.
- 6) Do not perform the assay under direct sunlight

3. Precautions for Disposal

- 1) Before disposal, used samples and their containers must be immersed in sodium hypochlorite solution at a concentration of greater than 0.1% for longer than 1 hour or autoclaved at 121°C for 20 minutes.
- 2) To prevent infections from spilled samples or solutions containing samples, wipe the spilled area thoroughly with disinfectants such as sodium hypochlorite solution at a concentration of greater than 0.1%.
- 3) The reagents and treated samples should be discarded as medical waste or industrial waste according to the waste disposal regulations.
- 4) The reagents should be disposed of in accordance with the Water Pollution Control act or related regulations.
- 5) HbA1c Reagent 1 contains sodium azide. It can react with lead or copper pipes to produce the highly explosive metal azide. Therefore, the reagent should be flushed with large amounts of water when disposing.

4. Other precautions

Do not use the containers for other purposes.

Storage and Shelf Life *

Name		Storage temperature	Shelf life
NORUDIA N HbA1c	HbA1c Reagent 1	2–10°C with protection from light	1 year from the date of manufacture
	HbA1c Reagent 2	2–10°C	

(The expiration date is printed on the outer package.)

Packaging

Name		Package
NORUDIA N HbA1c	(1) HbA1c Reagent 1	2 × 30 mL
	(2) HbA1c Reagent 2	2 × 10 mL

Constituent reagents are available in other configurations. For further details please contact SEKISUI MEDICAL CO., LTD.

Options

Name	Package
HbA1c Pretreatment Solution for NORDIA N HbA1c	2 × 200 mL

Shelf life: 1 year from the date of manufacture
This product is also available in different packages.
Please contact SEKISUI MEDICAL CO., LTD.

References

- 1) Edited by the Japan Diabetes Society: Treatment Guide for Diabetes 2010, 9, Bunkodo, 2010.
- 2) Wieland Hoelzel, Cas Weykamp, et al.: Clin Chem, 50, 166–174, 2004.
- 3) Atsunori Kashiwagi, et al.: Diabetol. Int. 3(1), 8–10, 2012.
- 4) In house data, SEKISUI MEDICAL CO., LTD.

Contact

SEKISUI MEDICAL CO., LTD.