



## Hemoglobin A1c Test Kit

**Banalyst HbA1c****Intended use**

Measurement of hemoglobin A1c (HbA1c) in human blood.

**Clinical importance**<sup>1)</sup>

Hemoglobin A1c (HbA1c, glycohemoglobin) is a stable type glycation product in which glucose is bonded to valine at the N-terminal of  $\beta$  chains of hemoglobin A. The production level of HbA1c depends upon the blood glucose level, and increases in proportion to the blood glucose level. Since the lifespan of HbA1c depends upon the lifespan of red blood cells (120 days), the measurement reflects the blood glucose level over the past 1 or 2 months. Therefore, the measurement value of HbA1c is used as an index of long term blood glucose control.

**Measurement principle**

## 1. Measurement principle

Latex agglutination immuno-turbidimetric method

The concentrations of HbA1c are measured using a latex agglutination immuno-turbidimetric assay for red blood cells. In more detail, red blood cells are separated and hemolyzed in the reagent chip, and the total hemoglobin including HbA1c is adsorbed onto the surface of latex particles. When anti-human hemoglobin A1c mouse monoclonal antibodies interacts with the adsorbed HbA1c (antigen-antibody reaction), aggregates (latex-HbA1c-anti-human hemoglobin A1c mouse monoclonal antibody complexes) are formed. The aggregates are measured as turbidity at 635 nm wavelength and the concentration of HbA1c is obtained using a standard curve.

## 2. Reaction flow in the reagent chip

All measurement steps (red blood cell separation → hemolysis → reaction (reagent dosing, mixing, and stirring) → measurement) are completed in a reagent chip. The reaction flow in the reagent chip is shown below.

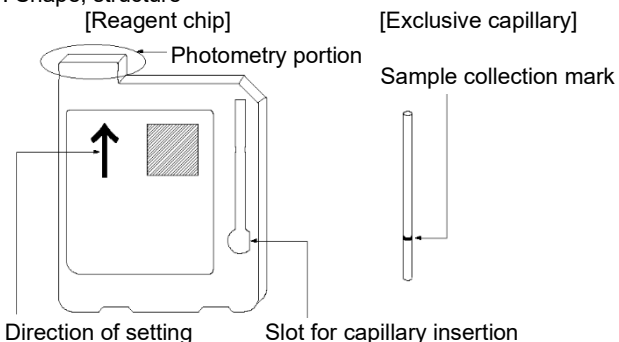
- [1] The whole blood collected in an exclusive capillary is transferred into a reagent chip, and red blood cells are separated by centrifugation here.
- [2] A certain amount of red blood cells are mixed with hemolysis reagent to prepare measurement sample.
- [3] The measurement sample and latex reagent are mixed and stirred together, and the total hemoglobin including HbA1c is adsorbed onto the surface of latex particles.
- [4] The latex particles with HbA1c adsorbed on the surface and anti-human hemoglobin A1c mouse monoclonal antibodies are mixed and stirred together, and latex aggregates are formed.
- [5] The aggregates are transferred to the photometry portion where absorbance is measured at 635 nm wavelength.
- [6] The aggregates are measured as turbidity here and the concentration of HbA1c is obtained using a standard curve.
- [7] The measured concentrations are expressed in "mmol/mol" and "%".

**Reference substance for calibration**

JCCRM 411

**Shape, structure, etc. (Kit constitution)**

## 1. Shape, structure



- Reagent chip (50 mm (H) × 40 mm (W) × 4.5 mm(D))

- Exclusive capillary (33 mm (L) × 1.8 mm (OD))

## 2. Kit constitution

Name	Component involved in reaction system	Quantity
Reagent chip	Anti-human hemoglobin A1c mouse monoclonal antibody	25 pieces
Exclusive capillary (accessory)	-	26 pieces

**Method of storage, expiration date**

## 1. Method of storage

To be refrigerated (2 °C to 8 °C, 36 °F to 46 °F)

## 2. Effective period

12 months after production

## 3. Expiration date (Exp.)

Written on the outer case, the aluminum bag, and the reagent chip

**General precautions**

- The reagent chips are in-vitro diagnostics. Do not use them for other purposes.
- Use the exclusive instrument (Banalyst) to measure reagent chips. Measurement is not possible with other instruments.
- For the use of reagent chip, follow the instructions on this paper and on the manuals provided with the instrument. Performance and measurement results cannot be guaranteed for use other than what is described.
- Do not freeze the reagent chips. If a reagent chip once frozen is used, correct measurement values cannot be obtained.
- The reagent in the reagent chips contains sodium azide as a preservative (content: 0.01 %). Do not disassemble reagent chips. If you get the reagent in your eyes or mouth or on your skin, flush them immediately with large amounts of water and receive medical treatment if necessary.

**Precautions in use or handling**

1. Pay special attention to avoid infection from a sample. Wear protective gloves during sample handling, for example.
2. Take a reagent chip out of the refrigerator and leave it in the aluminum bag until it warms up to ambient temperature. Take the reagent chip out of the aluminum bag immediately before use.
3. Store the reagent chips with the top of the outer case facing up. Do not turn the case upside down nor sideways.
4. Store the reagent chips in the method of storage described above. And do not use an expired reagent chip.
5. Repetition of refrigeration and leaving at ambient temperature shortens the shelf life of reagent chips. Avoid handling them in that way.
6. Do not freeze the reagent chips. And do not use a reagent chip once frozen.
7. Pay attention not to touch the photometry portion of a reagent chip.
8. Pay full attention in handling of reagent chips not to give strong impact such as dropping and shaking. Do not use any reagent chips damaged due to strong impact.
9. Make sure that the sample drawn into an exclusive capillary exceeds the sample collection mark. If the sample falls short of the mark, the measurement cannot be correct.
10. Pay attention not to soil the two-dimensional code of a reagent chip.
11. If an error occurs in reading a two-dimensional code, follow the instructions on the manuals provided with the instrument.
12. Immediately after the sample draw, insert the exclusive capillary into the reagent chip and start measurement quickly.
13. If an error occurs during measurement, measure the sample again.
14. The reagent in the reagent chips contains sodium azide as a preservative. Handle it appropriately not to form explosive

metal azide when disposing of a reagent chip.

15. Dispose of the reagent chip after use following the national and regional regulations relating to wastes.
16. Reagent chips are for one use only.

#### ■Precautions in operation■

##### 1. Sample properties and sample collecting

- Use whole blood as measurement sample.
- When whole blood with hematocrit of 25 % or less is used, correct measurement value may not be obtained.
- A minimum of 4  $\mu\text{L}$  sample is required for the test.
- Draw the sample into the exclusive capillary through the end closest to the sample collection mark until the sample exceeds the mark. Even if the sample drawn through the opposite end, the measurement value is not influenced.
- Measure the sample immediately after the sample draw.
- When collecting the measurement sample from the fingertip or earlobe, avoid direct contact of the exclusive capillary with the sampling site. And do not squeeze or milk the blood from the sampling site in capillary blood sampling.
- When the blood collected in a blood collection tube is used as a measurement sample, invert the tube to mix the blood well before drawing the sample into an exclusive capillary.
- The presence of other substances in the test sample may infrequently cause to give values higher (nonspecific reaction) or lower (interfering reaction) than the correct value.

##### 2. Interfering substances

- The measurement values are not influenced by the presence of up to 1410 FTU (formazin turbidity) of chyle, up to 21 mg/dL of free bilirubin, up to 20 mg/dL of conjugated bilirubin, up to 55 IU/mL of rheumatoid factor, and up to 50 mg/dL of ascorbic acid.
- The measurement values are not influenced by the normal usage of anticoagulants (heparin or EDTA) and glycolytic inhibitor (NaF).
- The whole blood collected using citric acid as an anticoagulant cannot be used as a sample. Also the whole blood collected in a blood collection tube containing citric acid in the glycolytic inhibitor (NaF) cannot be used as a sample.
- The evaluation of influences of mutant hemoglobin, acetylated hemoglobin, and carbamylated hemoglobin on the measurement value has not been established. When examining samples containing them, evaluate the examination results together with the results obtained using other methods.

#### ■Direction for use and dosage (operation method)■

1. Take a reagent chip out of the refrigerator and leave it in the aluminum bag for about 10 minutes until it warms up to ambient temperature. Use the reagent chip immediately after opening the aluminum bag.
2. Bring an end of the accompanying exclusive capillary with the sample to draw the sample until it exceeds the sample collection mark.
3. Immediately after the sample draw, insert the exclusive capillary into the reagent chip.
4. Put the reagent chip with the capillary inserted in place of the instrument quickly.
5. Press the "START" button of the instrument to start measurement.
6. The measurement results are displayed on the instrument after the measurement is completed.
7. Remove the used reagent chip from the instrument and dispose of it as a medical waste following the relevant national and regional regulations.

[Note] For more information on the instrument operations, refer to the manuals provided with the instrument.

#### ■Judgment of measurement results■

Have a physician-in-charge execute comprehensive clinical diagnosis based on the measurement results together with clinical conditions and other test results.

#### ■Performance■

##### 1. Performance

###### (1) Sensitivity

- [1] When normal saline is measured, the measurement results are 4.65 mAbs or lower.
- [2] When an administrative test substance of 10.9% is measured, the measurement results are 0.1 Abs or higher.

##### (2) Accuracy

When administrative test substances of specific concentrations are measured, the displayed values are within  $\pm 20\%$ .

##### (3) Within-run reproducibility

When administrative test substances of specific concentrations are measured repeatedly 5 times, the coefficient of variation is 10 % or less.

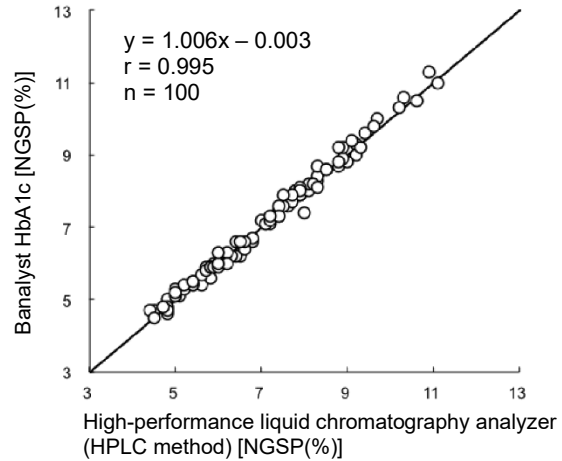
##### (4) Measurement range

3.3 % to 12.6 %

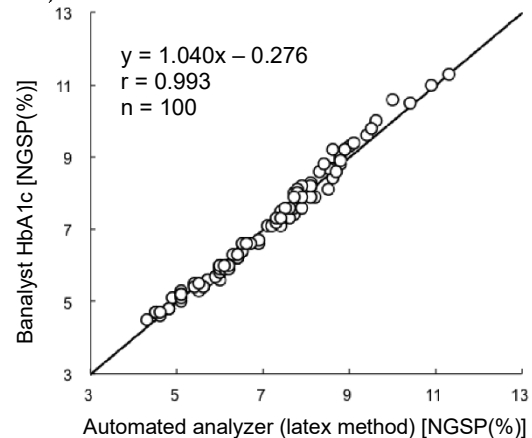
##### 2. Correlativity test result

Correlativity between this product and existing product was studied and good correlativity was obtained.

###### (1) Correlativity with general-purpose automated analyzer (HPLC method)



###### (2) Correlativity with general-purpose automated analyzer (latex method)



#### ■Reference value■

##### 1. Reference value<sup>2)</sup>

4 % to 6 %


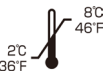










##### 2. Others

- When the measurement value is lower than the measurement limit (3.3 %), "Low" is displayed. When the measurement value is higher than the upper limit of measurement (12.6 %), "High" is displayed.
- When a measurement value is displayed as "Low" or "High", confirm the measurement value using other measurement methods.

#### ■Main literature■

1. Sacks DB et al., Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus. Clin Chem 2002; 48: 436-472.
2. Little RR et al., Relationship of glycosylated hemoglobin to oral glucose tolerance. Implications for diabetes screening. Diabetes 1988; 37(1): 60-64.

■Symbols■

	This way up		Temperature limitation
	Handle with care		Do not freeze
	Manufacturer		Consult instructions for use
	Use by		<i>In vitro</i> diagnostic medical device
	Batch code		Product code
	Do not reuse		Contents



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