

ⓄⓃⓄHbA1c IS*

Diagnostic reagent for quantitative in vitro determination of hemoglobin A1c in whole blood on InnovaStar®

Order Information

Cat. No.	
1 3329 99 10 760	100 determinations + 10 x 1800 µL Cleaner (Article number 970112) + 1 x ParamCard (Article number 970116)
970 100	InnovaStar® (instrument)
970 113	10 x 100 sample cups InnovaStar® 10/500
920 709	10 x 100 open-end capillaries 10 µL (heparinized)
970 115	300 mL System solution InnovaStar®

Summary [1,2,12]

Hemoglobin A1c (HbA1c) is a glycosylated hemoglobin which is formed by the non-enzymatic reaction of glucose with native hemoglobin. This process runs continuously throughout the circulatory life of the red cell (average life time 100 - 120 days). The rate of glycation is directly proportional to the concentration of glucose in the blood. The blood level of HbA1c represents the average blood glucose level over the preceding 6 to 8 weeks (due to the kinetics of erythrocyte turnover this period is more affected by the blood glucose level than the preceding weeks). Therefore, HbA1c is suitable for retrospective long-term monitoring of blood glucose concentration in individuals with diabetes mellitus. Clinical studies have shown that lowering of HbA1c level can help to prevent or delay the incidence of late diabetic complications. Besides, HbA1c determination can be used as an aid for diagnosis of diabetes mellitus.

As the amount of HbA1c also depends on the total quantity of hemoglobin the reported HbA1c value is indicated as a percentage of the total hemoglobin concentration.

Falsely low values (low HbA1c despite high blood glucose) may occur in people suffering from conditions with shortened red blood cell survival (hemolytic diseases) or significant recent blood loss (higher fraction of young erythrocytes). Falsely high values (high HbA1c despite normal blood glucose) have been reported in iron deficiency anemia (high proportion of old erythrocytes). These circumstances have to be considered in clinical interpretation of HbA1c values.

Method

Particle enhanced immunoturbidimetric test
HbA1c is determined directly without measurement of total hemoglobin.

Principle

Total Hb and HbA1c in hemolyzed blood bind with the same affinity to particles in R1. The amount of binding is proportional to the relative concentration of both substances in the blood.

Mouse anti-human HbA1c monoclonal antibody (R2) binds to particle bound HbA1c. Goat anti-mouse IgG polyclonal antibody (R3) interacts with the monoclonal mouse anti-human HbA1c antibody and agglutination takes place. The measured absorbance is proportional to the HbA1c bound to particles, which in turn is proportional to the percentage of HbA1c in the sample.

Standardization

The assay is standardized according to the approved IFCC reference method [3].

NGSP and IFCC values show a linear relationship and, therefore, can be calculated from each other using the following equations:

$$\text{HbA1c (IFCC}^{\text{b}}) = (\text{HbA1c (NGSP}^{\text{a}}) - 2.15) / 0.0915$$

$$\text{HbA1c (NGSP}^{\text{a}}) = 0.0915 \times \text{HbA1c (IFCC}^{\text{b}}) + 2.15$$

a: NGSP values in %

b: IFCC values in mmol/mol

IFCC: International Federation of Clinical Chemistry [3,4,9]

DCCT: Diabetes Control and Complications Trial [5]

NGSP: National Glycohemoglobin Standardization Program [6]

HbA1c and Average Glucose concentrations [10]

Due to a linear correlation between hemoglobin A1c and average glucose concentrations, HbA1c values can be converted in estimated average glucose values by means of the following equations:

Standardization according to IFCC (calculated referring to literature reference 10):

$$\text{Average glucose conc. [mg/dL]} = 2.63 \times \text{HbA1c}^{\text{b}} + 15.01$$

$$\text{Average glucose conc. [mmol/L]} = 0.146 \times \text{HbA1c}^{\text{b}} + 0.829$$

b: HbA1c values in mmol/mol IFCC

Standardization according to NGSP:

$$\text{Average glucose concentration [mg/dL]} = 28.7 \times \text{HbA1c}^{\text{a}} - 46.7$$

$$\text{Average glucose concentration [mmol/L]} = 1.59 \times \text{HbA1c}^{\text{a}} - 2.59$$

a: HbA1c values in % NGSP

No significant differences in the regression equation were observed for variations in individuals tested, including sex, presence or absence of diabetes, type of diabetes, age, race, and ethnicity. Although this equation can be used for the majority of individuals, each laboratory has to reassess itself if the regression equations mentioned are applicable for the patient group to be examined.

Reagents

Components and Concentrations

R1:	Buffer	20 mmol/L
	Latex	0.14%
R2:	Buffer	10 mmol/L
	Mouse anti-human HbA1c monoclonal antibody	5.5 mg/dL
R3:	Buffer	10 mmol/L
	Goat anti-mouse IgG polyclonal antibody	67 mg/dL

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C and protected from light. Do not freeze reagents! Damaged or opened reagent cartridges must not be used!

Reagent Preparation

The reagents are ready to use. Bring reagent to room temperature. Make sure that the reagent is at the bottom of the cartridge.

Warnings and Precautions

1. Reagent 2 contains animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
2. In very rare cases, samples of patients with gammopathy might give falsified results [13].
3. Heterophile antibodies in patient samples may cause falsified results.
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
5. For professional use only!

Waste Management

Please refer to local legal requirements.

Specimen

Capillary whole blood (taken with heparinized capillary), whole blood collected with EDTA

Please collect whole blood by standard venipuncture and fill the blood collection tube according to manufacturer specifications.

Discard contaminated specimens.

Sample preparation

For sample preparation sample cups InnovaStar® 10/500 (magenta cups) and open-end capillaries (10 µL/heparinized) are required. Take the patient sample with open-end capillary as described in the user manual. Put the filled capillary in the sample cup. Mix the sample and start the measurement directly.

Specimen stability [7]:

Whole blood	1 week	at	2 – 8°C
Hemolysate	10 hours	at	15 - 25°C
Hemolysate	10 days	at	2 – 8°C

Assay Procedure

Application is read by the ParamCard (see user manual InnovaStar®)

For every measurement of HbA1c place a cup with cleaner (orange cup) in the last position of the slider.

Calibration and Calculation

The calibration is stored on the ParamCard which is included in the reagent kit and is read after the receipt of the reagent (see user manual InnovaStar®). The calibration stability is 9 months. Fourteen days before the recalibration date, the instrument points to recalibration. This requires that a lot-specific code for the registration of a recalibration curve is entered into the instrument. The procedure of entering the code is described in the user manual for InnovaStar®. The successful entry of the recalibration curve has to be verified by the measurement of controls. The calibration was performed with IFCC calibrators and is therefore traceable to the approved IFCC reference method.

Controls

For internal quality control, a DiaSys TruLab HbA1c liquid control should be assayed every measuring day. An external quality control is recommended.

Each user should establish corrective action in case of deviation in control recovery.

	Cat. No.	Kit size
TruLab HbA1c liquid Level 1	5 9790 99 10 060	1 x 0.25 mL
TruLab HbA1c liquid Level 2	5 9800 99 10 060	1 x 0.25 mL

Performance Characteristics

Measuring Range

The test has a measuring range from 30 – 150 mmol/mol HbA1c according to IFCC (4.9 – 16% according to DCCT/NGSP). The assay is applicable for total hemoglobin concentrations in blood from 6.6 to 26 g/dL.

Interferences

The study on interferences was conducted according to CLSI protocol EP7-A2.

IFCC

For each interfering substance two samples with different HbA1c values have been tested; a low-level sample within a HbA1c range of 20 – 40 mmol/mol and a high level sample within a HbA1c range of 60 – 100 mmol/mol.

DCCT/NGSP

For each interfering substance, two samples with different HbA1c values have been tested; a low-level sample within a HbA1c range of 4.0 – 5.8% and a high level sample within a HbA1c range of 7.6 – 11.3%.

The table below summarizes the results, which comply for all tested levels using IFCC as well as DCCT/NGSP standardization.

Interfering substance	Interferences < 7% DCCT/NGSP and < 10% IFCC
Ascorbate	up to 60 mg/dL
Bilirubin (conjugated and unconjugated)	up to 60 mg/dL
Glucose	up to 1000 mg/dL
Hemoglobin, acetylated	up to 10 mmol/L
Hemoglobin, carbamylated	up to 10 mmol/L
Lipemia (triglycerides)	up to 2000 mg/dL
N-acetylcysteine (NAC)	up to 1500 mg/L
Urea	up to 300 mg/dL
Rheumatoid factor	up to 500 IU/mL
No interference is observed by Schiff base (labile intermediates) [7]. Alcoholism and ingestion of large doses of aspirin may lead to implausible results. For further information on interfering substances, refer to Young DS [11].	

Hemoglobin variants [7]:

The variants AS, AC, AD, AG, DD and elevated A2 showed no significant interferences.

The variants AE, AJ, SS, CC, SC, SE, EE, elevated F and elevated A2/F can lead to deviant HbA1c results (> 10% IFCC; > 7% DCCT/NGSP).

Sensitivity/Limit of Detection

The limit of detection is 30 mmol/mol HbA1c (4.9% HbA1c DCCT/NGSP).

Precision (Values according to DCCT/NGSP)			
Within run (n=20)	Sample 1	Sample 2	Sample 3
Mean [% DCCT/NGSP]	5.58	7.63	10.8
Coefficient of variation [%]	0.985	1.27	1.77
Day to day (n=20)	Sample 1	Sample 2	Sample 3
Mean [% DCCT/NGSP]	5.46	7.23	10.8
Coefficient of variation [%]	0.892	1.19	1.05
Total precision (CLSI) (n=80)	Sample 1	Sample 2	Sample 3
Mean [% DCCT/NGSP]	5.41	7.19	10.7
Coefficient of variation [%]	2.28	1.90	2.07

Method Comparison

A comparison of DiaSys oneHbA1c IS (y) with a HPLC assay (x) using 100 samples gave following results (DCCT/NGSP values):

$$y = 1.08x - 0.435\%; r = 0.997$$

Reference Range

Suggested target values for HbA1c: [8]

	mmol/mol	%
Non-diabetics	20 – 42	4 – 6
Target of therapy	< 53	< 7
Change of therapy	> 64	> 8

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

HbA1c cut point value for diagnosis of diabetes mellitus [12]:

According to a recommendation of the American Diabetes Association (ADA): ≥ 6.5% (NGSP) (48 mmol/mol (IFCC))

Patients with HbA1c values in the range of 5.7 – 6.4% HbA1c (NGSP) or 39 – 46 mmol/mol HbA1c (IFCC) may be at high risk of developing diabetes.

Literature

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Manufacturer

DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany

