oneHbA1c FS*

Diagnostic reagent for quantitative in vitro determination of hemoglobin A1c in whole blood on photometric systems

Order Information

Cat. No.	Kit size
1 3329 99 10 930	R1 3 x 20 mL + R2 2 x 10 mL + R3 1 x 10 mL
1 3329 99 10 935	R1 2 x 15 mL + R2 1 x 10 mL + R3 1 x 5 mL
1 3329 99 90 380	R1 2 x 15 mL + R2 1 x 10 mL + R3 1 x 5 mL
1 4570 99 10 113	1 x 500 mL oneHbA1c Hemolyzing Solution

Summary [1, 2, 12]

Hemoglobin A1c (HbA1c) is a glycated 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 testing may be used 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 with 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 and Principle

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

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 can therefore be calculated from each other using the following equation:

HbA1c *(IFCC[a])* = (HbA1c *(NGSP[b])* – 2.15) / 0.0915
HbA1c *(NGSP[b])* = 0.0915 x HbA1c *(IFCC[a])* + 2.15

a: IFCC values in mmol/mol
b: NGSP values in %

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):

Average glucose conc. [mg/dL] = 2.63 x $HbA1c^a$ + 15.01
Average glucose conc. [mmol/L] = 0.146 x $HbA1c^a$ + 0.829

a: HbA1c values in mmol/mol IFCC

Standardization according to NGSP:
Average glucose concentration [mg/dL] = 28.7 x $HbA1c^b$ – 46.7
Average glucose concentration [mmol/L] = 1.59 x $HbA1c^b$ – 2.59

b: 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 reassure 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 contamination is avoided. Do not freeze the reagents and protect them from light.

Reagent Preparation

Reagent 1 is ready to use. Reagent 2 and reagent 3 must be premixed before use. Transfer 5 mL of R3 into one bottle R2 and mix well immediately.
Stability of premixed R2/R3: One month stored at 2 – 8°C.

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. Immediately after HbA1c measurement cleaning of cuvettes is necessary. Use the alkaline cuvette washing solution which is recommended by the analyzer manufacturer.
6. For professional use only!

Waste Management

Please refer to local legal requirements.

Materials Required

General laboratory equipment

Specimen

Whole blood collected with EDTA
Discard contaminated specimens.
Please collect whole blood by standard venipuncture and fill the blood collection tube according to manufacturer specifications.

Sample Preparation:

For sample preparation the DiaSys oneHbA1c Hemolyzing Solution Cat. No 1 4570 99 10 113 is required.

Sample preparation:

Hemolyzing Solution	1000 µL
Sample/Calibrator/Control	20 µL

Mix and allow to stand for 5 minutes or until complete lysis is apparent.

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 sheets for automated systems are available on request.

Wavelength	660 nm
Optical path	1 cm
Temperature	37°C
Measurement	Against air

* Fluid Stable

2-component system - premixed R2/R3

Sample or calibrator	15 µL
Reagent 1	600 µL
Mix, incubate for 5 min., then add:	
Reagent 2/3	300 µL
Mix, read absorbance after **exactly** 1 min and read absorbance after a total of **exactly** 5 min.	

Calculation

The concentration of HbA1c in unknown samples is derived from a calibration curve using an appropriate mathematical model such as e.g. spline. The calibration curve is obtained with 4 calibrators at different levels and NaCl solution (9 g/L) for determination of the zero value.

Stability of calibration: 2-component system 4 weeks

Calibrators and Controls

DiaSys TruCal HbA1c liquid is recommended for calibration The calibrator values have been made traceable to the approved IFCC reference method. Values according to DCCT/NGSP in % have been derived from the values according to IFCC by calculation. Use DiaSys TruLab HbA1c liquid for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal HbA1c liquid	1 3320 99 10 043	4 x 0.25 mL
TruLab HbA1c liquid Level 1	5 9790 99 10 074	4 x 0.25 mL
TruLab HbA1c liquid Level 2	5 9800 99 10 074	4 x 0.25 mL

Performance Characteristics

Data evaluated on Hitachi 917

Measuring Range

The test has been developed to determine concentrations of HbA1c within a measuring range from 30 – 150 mmol/mol according to IFCC (4.9 – 16% according to NGSP), at least up to the concentration of the highest calibrator.
The assay is applicable for 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 for the 2-component system.

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 1000 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.

Sensitivity/Limit of Detection

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

Precision

(Hitachi 917, 2-component system; Values according to IFCC)

Within-run precision n = 20	Mean [mmol/mol]	SD [mmol/mol]	CV [%]
Sample 1	36.4	0.572	1.57
Sample 2	60.0	0.522	0.869
Sample 3	87.6	1.01	1.15

Between day precision n = 20	Mean [mmol/mol]	SD [mmol/mol]	CV [%]
Sample 1	34.9	0.678	1.94
Sample 2	53.9	0.953	1.77
Sample 3	86.6	0.920	1.06

Method Comparison

A comparison of DiaSys oneHbA1c FS (y) to a commercially available assay (x) using 88 samples gave following results (IFCC values):
y = 1.01 x – 1.10 mmol/mol; r = 0.997

A comparison of DiaSys oneHbA1c FS (y) to a HPLC assay (x) using 100 samples gave following results (IFCC values):
y = 1.01 x – 0.437 mmol/mol; r = 0.997

Reference Range

Suggested target values for HbA1c [8]:

	mmol/mol IFCC	% NGSP
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

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 142-48.
2. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders Company; 1999. p. 790-6.
3. Jeppsson JO, Kobold U, Barr J, Finke A et al. Approved IFCC reference method for the measurement of HbA1c in human blood. Clin Chem Lab Med 2002; 40: 78–89.
4. Hoelzel W, Weykamp C et al. IFCC Reference System for Measurement of Hemoglobin A1c in Human Blood and the National Standardization Schemes in the United States, Japan, and Sweden: A Method-Comparison Study. Clin Chem 2004; 50 (1): 166-74.
5. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes in the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med.1993; 329: 977-86.
6. Little RR, Rohlfing CL, Wiedmeyer HM, Myers GL et al. The National Glycohemoglobin Standardization Program: A Five-Years Progress Report. Clin Chem 2001; 47: 1985-92.
7. Data on file at DiaSys Diagnostic Systems GmbH.
8. Panthegini M, John WG on behalf of the IFCC Scientific Division. Implementation of haemoglobin A1c results traceable to the IFCC reference system: the way forward. Clin Chem Lab Med 2007; 45(8): 942-4.
9. Nordin G., Dybkær R. Recommendation for term and measurement unit for "HbA1c". Clin Chem Lab Med 2007; 45(8): 1081-2.
10. Sacks DB. Translating Hemoglobin A1c into Average Blood Glucose: Implications for Clinical Chemistry. Clinical Chemistry 2008; 54: 1756-8.
11. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
12. Sacks DB, Arnold M, Bakris GL, Bruns DE, AR Horvath et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem 2011; 57(6): e1-e47.
13. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240–1243.

Manufacturer

DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany