

ⓄⓃⓔ HbA1c FS*

Diagnostic reagent for quantitative in vitro determination of hemoglobin A1c (HbA1c) in whole blood on DiaSys respons[®]910

Order Information

Cat. No. 1 3329 99 10 922

4 twin containers (R1+R2) and 2 bottles (R3) for a total of 400 tests

Cat. No. 1 3329 99 10 927

2 twin containers (R1+R2) and 1 bottles (R3) for a total of 200 tests

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 [1].

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

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

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

a: NGSP values in %

b: IFCC values in mmol/mol

IFCC: International Federation of Clinical Chemistry [1,2,3]

DCCT: Diabetes Control and Complications Trial [4]

NGSP: National Glycohemoglobin Standardization Program [5]

HbA1c and Average Glucose Concentrations [6]

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):

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

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

b: HbA1c values in mmol/mol IFCC

Standardization according to NGSP:

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

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

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 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
	Stabilizers	

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, protected from light, evaporation and contamination is avoided. DiaSys respons containers provide protection from light. Do not freeze the reagents!

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.

Reagent Preparation

Reagent 2 and reagent 3 must be premixed before use. Transfer 3.5 mL R3 into the R2 cavity of the twin container. Mix very gently to avoid foaming.

Stability of premixed R2/R3: One month, stored at 2 – 8 °C.

With each bottle change of premixed reagents 2 and 3 a calibration must be done.

Specimen

Whole blood collected with EDTA

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

Sample preparation:

Sample preparation requires DiaSys oneHbA1c Hemolyzing Solution Cat. No 1 4570 99 10 113.

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

Discard contaminated specimens.

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

Measuring range from 30 to 150 mmol/mol HbA1c according to IFCC (4.9 to 16% according to DCCT/NGSP) at least up to the concentration of the highest calibrator. The assay is applicable for total hemoglobin concentrations in blood from 6.6 to 26 g/dL.	
Limit of detection**	30 mmol/mol HbA1c
On-board stability	4 weeks
Calibration stability	2 weeks

**according to CLSI Document EP17-A2, Vol. 32, No. 8

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 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 (> 10% IFCC; > 7% DCCT/NGSP).

Precision (Values according to IFCC)			
Within run (n=20)	Sample 1	Sample 2	Sample 3
Mean [mmol/mol]	32.2	57.5	87.2
Coefficient of variation [%]	1.86	1.20	1.57
Day to day (n=20)	Sample 1	Sample 2	Sample 3
Mean [mmol/mol]	33.4	54.8	89.8
Coefficient of variation [%]	3.81	3.79	3.69
Total precision (CLSI) (n=80)	Sample 1	Sample 2	Sample 3
Mean [mmol/mol]	32.8	53.5	85.5
Coefficient of variation [%]	4.74	4.45	4.68

Method comparison (n=100) according to IFCC	
Test x	HbA1c HPLC Arkray HA-8160 V7.41
Test y	DiaSys one HbA1c FS (respons [®] 910)
Slope	1.05
Intercept	-2.95 mmol/mol
Coefficient of correlation	0.996

Reference Range

Suggested target values for HbA1c [9]:

	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.

Reagent information

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

According to a recommendation of the American Diabetes Association (ADA): $\geq 6.5\%$ (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
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oneHbA1c FS - Linear kinetic

Application for hemolysate from whole blood samples

This application was set up and evaluated by DiaSys. It is based on the standard equipment at that time and does not apply to any equipment modifications undertaken by unqualified personnel.

Identification	
This method is usable for analysis:	Yes
Twin reaction:	No
Name:	HBA1C
Shortcut:	
Reagent barcode reference:	713
Host reference:	

Technic	
Type:	Fixed time kinetic
First reagent:[μ L]	144
Blank reagent	Yes
Sensitive to light	
Second reagent:[μ L]	72
Blank reagent	No
Sensitive to light	
Main wavelength:[nm]	660
Secondary wavelength:[nm]	
Polychromatic factor:	
1 st reading time [min:sec]	05:00
Last reading time [min:sec]	10:00
Reaction way:	Increasing
Linear Kinetics	
Substrate depletion: Absorbance li	
Linearity: Maximum deviation [%]	
Fixed Time Kinetics	
Substrate depletion: Absorbance limit	
Endpoint	
Stability: Largest remaining slope	
Prozone Limit [%]	

Reagents	
Decimals	
Units	

Sample	
Diluent	DIL A (NaCl)
Hemolysis:	
Agent [μ L]	0 (no hemolysis)
Cleaner	
Sample [μ L]	0
Technical limits	
Concentration technical limits-Lower	30
Concentration technical limits-Upper	150
SERUM	
Normal volume [μ L]	4**
Normal dilution (factor)	1**
Below normal volume [μ L]	10**
Below normal dilution (factor)	1**
Above normal volume [μ L]	3**
Above normal dilution (factor)	1**
URIN	
Normal volume [μ L]	4**
Normal dilution (factor)	1**
Below normal volume [μ L]	10**
Below normal dilution (factor)	1**
Above normal volume [μ L]	3**
Above normal dilution (factor)	1**
PLASMA	
Normal volume [μ L]	4**
Normal dilution (factor)	1**
Below normal volume [μ L]	10**
Below normal dilution (factor)	1**
Above normal volume [μ L]	3**
Above normal dilution (factor)	1**
CSF	
Normal volume [μ L]	4**
Normal dilution (factor)	1**
Below normal volume [μ L]	10**
Below normal dilution (factor)	1**
Above normal volume [μ L]	3**
Above normal dilution (factor)	1**
Whole blood	
Normal volume [μ L]	4**
Normal dilution (factor)	1**
Below normal volume [μ L]	10**
Below normal dilution (factor)	1**
Above normal volume [μ L]	3**
Above normal dilution (factor)	1**

Results	
Decimals	2
Units	mmol/mol
Correlation factor-Offset	0.000
Correlation factor-Slope	1.000

Range	
Gender	All
Age	
SERUM	>=20 <=42
URINE	
PLASMA	
CSF	
Whole blood	
Gender	
Age	
SERUM	
URINE	
PLASMA	
CSF	
Whole blood	

Contaminants	
Please refer to r910 Carryover Pair Table	

Calibrators details	
Calibrator list	Concentration
Cal. 1/Blank	0
Cal. 2	*
Cal. 3	*
Cal. 4	*
Cal. 5	*
Cal. 6	
	Max delta abs.
Cal. 1	0.0100
Cal. 2	0.0100
Cal. 3	0.0250
Cal. 4	0.0500
Cal. 5	0.0600
Cal. 6	
Drift limit [%]	0.8

Calculations	
Model	Akima Spline
Degree	Auto

* Enter calibrator value

**Information has to be inserted (required by software), although HbA1c samples must not be diluted!