respons®910

HbA1c net FS*

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

Order Information

Cat. No. 1 3348 99 10 921
4 twin containers for 100 tests each
Cat. No. 1 3348 99 10 920
4 twin containers for 200 tests each

Method

Hemoglobin: Photometric test
HbA1c: Colorimetric, enzymatic method

Principle

The concentrations of HbA1c and hemoglobin are determined separately and are used to calculate the HbA1c ratio from total hemoglobin.

Hemoglobin measurement
Whole blood samples are lysed with hemolyzing solution. Hemoglobin is released from the erythrocytes. The absorbance of hemoglobin is measured at 570 nm after addition of reagent R1 and is proportional to the total hemoglobin concentration in the sample.

HbA1c measurement [16]
After addition of R2, fructosylated dipeptides from the N-terminal part of the hemoglobin β-chain are released by protease. Hydrogen peroxide (H_2O_2) is produced by oxidative cleavage of fructosylated dipeptides by FPOX (fructosyl peptide oxidase). The H_2O_2 generated is determined colorimetrically by reaction with a chromogen in presence of peroxidase at 660 nm. The absorbance increase is proportional to the HbA1c concentration.

Standardization

The assay is standardized according to IFCC [1] and DCCT/NGSP [4] reference methods. Calculation of patient and control values is possible according to IFCC [mmol/mol] as well as according to DCCT/NGSP [%].

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

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

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

IFCC: International Federation of Clinical Chemistry [1,2,7]
DCCT: Diabetes Control and Complications Trial [3]
NGSP: National Glycohemoglobin Standardization Program [4]

HbA1c and Average Glucose Concentrations [8]

Due to a linear correlation between hemoglobin A1c and average glucose concentrations, HbA1c values can be converted into estimated average glucose values by means of the following equations:
Standardization according to IFCC (calculated referring to literature reference 8):

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

b: HbA1c values in mmol/mol IFCC

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

a: HbA1c values in % NGSP

No significant differences in the regression equation were observed for variations in individuals tested regarding 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 verify whether the regression equations mentioned are applicable for the patient group to be examined.

Reagents

Components and Concentrations

R1:	Buffer	100 mmol/L
	FPOX	≥ 0.5 kU/L
	Ethlyene glycol derivative	< 10%
R2:	Buffer	20 mmol/L
	Protease	≥ 500 kU/L
	Chromogen	≥ 0.05 mmol/L
	Ethlyene glycol derivative	< 10%

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. **Instrument software as of version 4.8.8.1 is mandatory!**
2. Reagent 1 contains animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
3. Hemoglobin and HbA1c values in g/dL determined with DiaSys HbA1c net FS are used to calculate the HbA1c ratio from total hemoglobin exclusively. Individual results for hemoglobin and HbA1c must not be used for diagnostic purposes.
4. Falsely low values (low HbA1c despite high blood glucose) may occur in people with conditions such as shortened red blood cell survival (e.g. hemolytic diseases) or significant recent blood loss during the weeks before (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. Care must also be taken in clinical interpretation of HbA1c results from patients with hemoglobin variants.
5. In very rare cases, samples of patients with gammopathy might give falsified results [15].
6. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
7. Refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, results should always be assessed with patient`s medical history, clinical examinations and other findings.
8. For professional use only!

Waste Management

Please refer to local legal requirements.

Materials Required but not Provided

General laboratory equipment

Reagent Preparation

The reagents are ready to use. The bottles are placed directly into the reagent rotor.

Homogenize HbA1c net Hemolyzing Solution by repeated inversion. Due to composition of the hemolyzing solution an opalescent and slightly turbid appearance remains. Avoid foaming! Do not shake!

Specimen

Whole blood collected with EDTA

Specimen Stability [5]:

Whole blood	1 week	at	2 – 8°C

Discard contaminated specimens

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

Subsequent blood collection tubes have been tested:

BD Vacutainer (Art. No. 368856), 3.0 mL, K2EDTA
Sarstedt Monovette (Art. No. 05.1167), 2.7 mL, K3EDTA

DiaSys measurements have been performed on day of blood collection. The samples showed a normal blood sedimentation rate.

Per run, determination of up to 30 whole blood samples in primary tubes is possible if:

- Calibration and control recovery have previously been done in a separate run.
- Above-mentioned primary tubes are homogenized by swirling several times and analyzed immediately in one run to reduce the effects of erythrocyte sedimentation.
- Only HbA1c net is determined in this run.

Samples containing too little erythrocytes are flagged, no value is displayed. In this case, re-mix the sample and repeat the analysis, optionally via the STAT position.

Sample Preparation

DiaSys HbA1c net Hemolyzing Solution is required for sample preparation.

	Cat. No.	Kit Size
HbA1c net Hemolyzing Solution	1 4590 99 10 923	4 x 200 tests

The bottles of DiaSys HbA1c net Hemolyzing Solution are placed directly into the reagent rotor. Hemolysis is performed on board of the instrument automatically.

Note: TruCal HbA1c net Level 1 for onboard hemolysis is prepared by diluting the dissolved calibrator with aqua dest.

2 parts dist. water + 1 part TruCal HbA1c net Level 2

Use TruCal HbA1c net Level 1 immediately for analysis.

Calibration

The concentrations of HbA1c and hemoglobin in unknown samples are derived from linear calibration curves.
Each calibration curve is obtained with 2 calibrators at different levels without a zero value.

Calculation

Calculation of HbA1c ratio from total hemoglobin in mmol/mol is done by the instrument automatically according to IFCC standardization.

IFCC

$$\text{HbA1c [mmol/mol]} = \left(\frac{\text{HbA1c [g/dL]}}{\text{Hb [g/dL]}}\right) \times 1000$$

DCCT/NGSP

Values in percent according to DCCT/NGSP:

$$\text{HbA1c [\%]} = \left(91.5 \times \frac{\text{HbA1c [g/dL]}}{\text{Hb [g/dL]}}\right) + 2.15$$

Calibrators and Controls

DiaSys TruCal HbA1c net is recommended for calibration. The assigned values of TruCal HbA1c net have been made traceable to IFCC reference method [1]. DiaSys TruLab HbA1c net should be assayed for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal HbA1c net	1 3350 99 10 044	2 x 0.3 mL
TruLab HbA1c net Level 1	5 9930 99 10 076	6 x 1 mL
TruLab HbA1c net Level 2	5 9940 99 10 076	6 x 1 mL

Performance Characteristics

Measuring range from 20 – 150 mmol/mol according to IFCC (4 - 16% according to DCCT/NGSP). The assay is applicable for hemoglobin concentrations in blood from 6 – 30 g/dL (3.73 – 18.6 mmol/L).	
Limit of detection**: HbA1c Hemoglobin	 0.2 g/dL 1.5 g/dL
Onboard stability	4 weeks
Calibration stability	3 weeks (8 hours/day uncooled)
Calibration stability	1 week (24 hours/day uncooled)

** lowest measurable concentration which can be distinguished from zero
mean + 1.645 x SD (n = 60) of an analyte free specimen

Specificity/Interferences

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

IFCC
For each interfering substance, two samples with different hemoglobin and HbA1c values have been tested; a low level sample within a hemoglobin range of 7 – 10 g/dL and a HbA1c range within 25 – 45 mmol/mol and a high level sample within a hemoglobin range of 10 – 15 g/dL and a HbA1c range > 55 mmol/mol.

DCCT/NGSP
For each interfering substance, two samples with different hemoglobin and HbA1c values have been tested; a low level sample within a hemoglobin range of 7 – 10 g/dL and a HbA1c range within 4.0 – 6.3% and a high level sample within a hemoglobin range of 10 – 15 g/dL and a HbA1c range > 7.15%.

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

Interfering substance	Interferences < 10% (with hematocrit correction)	HbA1c [mmol/mol]
Ascorbate	up to 50 mg/dL	35.3
	up to 50 mg/dL	70.9
Bilirubin, conjugated	up to 10 mg/dL	35.4
	up to 10 mg/dL	63.7
Bilirubin, unconjugated	up to 10 mg/dL	36.3
	up to 10 mg/dL	65.9
Glucose	up to 1000 mg/dL	40.6
	up to 1000 mg/dL	66.2
Hemoglobin, acetylated	up to 10 mmol/L	34.6
	up to 10 mmol/L	70.6
Hemoglobin, carbamylated	up to 10 mmol/L	34.8
	up to 10 mmol/L	70.0
Lipemia (triglycerides)	up to 750 mg/dL	35.8
	up to 1000 mg/dL	72.8
N-acetylcysteine (NAC)	up to 2000 mg/L	42.3
	up to 2000 mg/L	71.3
Urea	up to 300 mg/dL	34.6
	up to 300 mg/dL	69.7
Uric acid	up to 20 mg/dL	36.9
	up to 20 mg/dL	76.9
Alcoholism and ingestion of large doses of acetylsalicylic acid may lead to im-plausible results. For further information on interfering substances refer to Young DS [10].		

Hemoglobin variants may lead to deviant HbA1c results: The tested Hemoglobin variants HbS, HbC, HbD, HbE, HbJ, HbG, HbSC, HbSE, HbEE and HbF showed no significant interference.

Hemoglobin Variant	Percentage of Hemoglobin Variant (≤)	Target Value Range HbA1c [% DCCT/NGSP]	Mean recovery HbA1c [%]
AS	40% S	5.2 – 8.8	94.7
AC	36% C	5.0 – 7.4	97.1
AD	41% D	5.6 – 7.0	93.9
AE	26% E	5.9 – 7.6	99.1
AJ	50% J	5.2 – 8.4	100
AG	20% G	6.1 – 6.6	97.4
SC	52% S, 44% C	4.5 – 7.0	91.6
SE	65% S, 27% E	7.4	95.4
EE	94% E	5.1 – 8.9	98.0
Elevated F	4.6% F	6.5 – 8.1	93.6

Precision Values according to IFCC			
Within run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mmol/mol]	32.9	34.8	63.6
Coefficient of variance [%]	2.27	2.69	2.14
Between run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mmol/mol]	31.0	33.2	62.6
Coefficient of variance [%]	2.46	3.31	2.59

Method comparison (n=100)	
Test x	HPLC assay
Test y	DiaSys HbA1c net FS respons®910
Slope	0.983
Intercept	1.62 mmol/mol
Coefficient of correlation	0.994

Reference Range

Suggested target values for HbA1c [6]:

	IFCC [mmol/mol]	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 [14]**:**

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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4. 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.
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8. Sacks DB. Translating Hemoglobin A1c into Average Blood Glucose: Implications for Clinical Chemistry. Clinical Chemistry 2008; 54: 1756-
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15. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240–1243.
16. Ferri S, Kim S, Tsugawa W, Sode K. Review of Fructosyl Amino Acid Oxidase Engineering Research: A Glimpse into the Future of Hemoglobin A1c Biosensing. Journal of Diabetes Science and Technology 2009; 3(3): 585-592.

IVD CE

Manufacturer

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respons®910

HbA1c net FS (reaction 1 hemoglobin)

Application for 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:	Yes
Name:	HbA1c net
Shortcut:	
Reagent barcode reference:	723
Host reference:	

Technic	
Type:	End point
First reagent:[µL]	150
Blank reagent	Yes
Sensitive to light	Yes
Second reagent:[µL]	
Blank reagent	
Sensitive to light	
Main wavelength:[nm]	570
Secondary wavelength:[nm]	800
Polychromatic factor:	1.000
1 st reading time [min:sec]	(04:24)
Last reading time [min:sec]	04:24
Reaction way:	Increasing
Linear Kinetics	
Linearity: Maximum deviation [%]	
Fixed Time Kinetics	
Endpoint	
Prozone Limit [%]	

Reagents	
Decimals	2
Units	g/dL

Sample	
Diluent	System water
Hemolysis:	
Agent [µL]	NET(R951) 200
Cleaner	CLN A (R900)
Sample [µL]	10
Technical limits	HbA1c [mmol/mol]: 20 -150
Concentration technical limits-Lower	6
Concentration technical limits-Upper	30
SERUM	
Normal volume [µL]	25
Normal dilution (factor)	1
Below normal volume [µL]	25
Below normal dilution (factor)	1
Above normal volume [µL]	25
Above normal dilution (factor)	1
URIN	
Normal volume [µL]	25
Normal dilution (factor)	1
Below normal volume [µL]	25
Below normal dilution (factor)	1
Above normal volume [µL]	25
Above normal dilution (factor)	1
PLASMA	
Normal volume [µL]	25
Normal dilution (factor)	1
Below normal volume [µL]	25
Below normal dilution (factor)	1
Above normal volume [µL]	25
Above normal dilution (factor)	1
CSF	
Normal volume [µL]	25
Normal dilution (factor)	1
Below normal volume[µL]	25
Below normal dilution (factor)	1
Above normal volume [µL]	25
Above normal dilution (factor)	1
Whole blood	
Normal volume [µL]	25
Normal dilution (factor)	1
Below normal volume[µL]	25
Below normal dilution (factor)	1
Above normal volume [µL]	25
Above normal dilution (factor)	1

Results	
Decimals	1
Units	mmol/mol
Correlation factor-Offset	0.000
Correlation factor-Slope	1.000

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

Contaminants	
Contaminant 1	
Wash with	
Cycle	
Volume [µL]	
Contaminant 2	
Wash with	
Cycle	
Volume [µL]	
Contaminant 3	
Wash with	
Cycle	
Volume [µL]	

Calibrators details	
Calibrator list	**Concentration**
Cal. 1/Blank	*
Cal. 2	*
Cal. 3	
Cal. 4	
Cal. 5	
Cal. 6	

	Max delta abs.
Cal. 1	0.005
Cal. 2	0.005
Cal. 3	
Cal. 4	
Cal. 5	
Cal. 6	
Drift limit [%]	0.8

Calculations	
Model	X
Degree	1

* Enter calibrator value

Calculation of HbA1c /Hb ratio is done automatically.

For values in percent according to DCCT/NGSP please enter 2.15 offset and a slope of 0.0915.

respons®910

HbA1c net FS (reaction 2 HbA1c)

Application for 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:	Yes
Name:	HbA1c net
Shortcut:	
Reagent barcode reference:	723
Host reference:	

Technic	
Type:	Fixed time kinetic
First reagent:[µL]	
Blank reagent	
Sensitive to light	
Second reagent:[µL]	50
Blank reagent	Yes
Sensitive to light	Yes
Main wavelength:[nm]	660
Secondary wavelength:[nm]	800
Polychromatic factor:	1.000
1 st reading time [min:sec]	5:00
Last reading time [min:sec]	10:00
Reaction way:	Increasing
Linear Kinetics	
Linearity: Maximum deviation [%]	
Fixed Time Kinetics	
Endpoint	
Prozone Limit [%]	

Reagents	
Decimals	3
Units	g/dL

Sample	
Diluent	System water
Hemolysis:	
Agent [µL]	NET(R951) 200
Cleaner	CLN A (R900)
Sample [µL]	10
Technical limits	HbA1c [mmol/mol]: 20 -150
Concentration technical limits-Lower	0.3
Concentration technical limits-Upper	2.0
SERUM	
Normal volume [µL]	25
Normal dilution (factor)	1
Below normal volume [µL]	25
Below normal dilution (factor)	1
Above normal volume [µL]	25
Above normal dilution (factor)	1
URIN	
Normal volume [µL]	25
Normal dilution (factor)	1
Below normal volume [µL]	25
Below normal dilution (factor)	1
Above normal volume [µL]	25
Above normal dilution (factor)	1
PLASMA	
Normal volume [µL]	25
Normal dilution (factor)	1
Below normal volume [µL]	25
Below normal dilution (factor)	1
Above normal volume [µL]	25
Above normal dilution (factor)	1
CSF	
Normal volume [µL]	25
Normal dilution (factor)	1
Below normal volume[µL]	25
Below normal dilution (factor)	1
Above normal volume [µL]	25
Above normal dilution (factor)	1
Whole blood	
Normal volume [µL]	25
Normal dilution (factor)	1
Below normal volume[µL]	25
Below normal dilution (factor)	1
Above normal volume [µL]	25
Above normal dilution (factor)	1

Results	
Decimals	1
Units	mmol/mol
Correlation factor-Offset	0.000
Correlation factor-Slope	1.000

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

Contaminants	
Contaminant 1	
Wash with	
Cycle	
Volume [µL]	
Contaminant 2	
Wash with	
Cycle	
Volume [µL]	
Contaminant 3	
Wash with	
Cycle	
Volume [µL]	

Calibrators details	
Calibrator list	**Concentration**
Cal. 1/Blank	*
Cal. 2	*
Cal. 3	
Cal. 4	
Cal. 5	
Cal. 6	

	Max delta abs.
Cal. 1	0.005
Cal. 2	0.015
Cal. 3	
Cal. 4	
Cal. 5	
Cal. 6	
Drift limit [%]	0.8

Calculations	
Model	X
Degree	1

* Enter calibrator value

Calculation of HbA1c /Hb ratio is done automatically.

For values in percent according to DCCT/NGSP please enter 2.15 offset and a slope of 0.0915.