BioMajesty

HbA1c net FS*

Diagnostic reagent for quantitative in vitro determination of hemoglobin A1c (HbA1c) in whole blood on BioMajesty JCA-BM6010/C

Order Information

Cat. No. 1 3348 99 10 962
R1: 6 x 300 tests
R2: 6 x 300 tests

Cat. No. 1 3348 99 10 964
R1: 2 x 160 tests
R2: 2 x 160 tests

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 exclusively.

Hemoglobin measurement
Whole blood samples are lysed with hemolyzing solution. Hemoglobin is released from the erythrocytes. The absorbance of hemoglobin is measured at 571 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 a 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 658 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 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 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 and contamination and evaporation are avoided. Do not freeze the reagents! Protect reagents from light!

Warnings and Precautions

1. Reagent 1 contains animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
2. 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.
3. 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.
4. In very rare cases, samples of patients with gammopathy might give falsified results [15].
5. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
6. Please 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.
7. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

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

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

Specimen Stability [5]:

Whole blood 1 week at 2 – 8°C

Discard contaminated specimens.

Sample Preparation:

For sample preparation the DiaSys HbA1c net Hemolyzing Solution is required.

	Cat. No.	Kit Size
HbA1c net Hemolyzing Solution	1 4590 99 10 967	4 x 450 tests
	1 4590 99 10 961	2 x 160 tests

The bottles of DiaSys HbA1c net Hemolyzing Solution are placed directly into the reagent trays.

Reagent tray position has to be defined as “Diluent position” in application for sample, control and calibrator. Position number must be between 1 and 45 respectively 1 and 50.

Hemolysis is performed on board of the instrument automatically. Whole blood collection tubes must not be higher than 75 mm; otherwise contamination may occur!
Processing in batch mode is recommended.

Calibrators, controls and samples have to be hemolyzed before use. Please refer to subsequent pipetting scheme for on-board hemolysis:

	Preparation			
	Calibrator Level 1	Calibrator Level 2	Control	Sample
TruCal HbA1c net Level 1	1.6 µL	-	-	-
TruCal HbA1c net Level 2	-	5 µL	-	-
TruLab HbA1c net Level 1 and Level 2 /Sample	-	-	5 µL	5 µL
Add:				
HbA1c net Hemolyzing solution	100 µL	100 µL	100 µL	100 µL

* fluid stable

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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

After entering the calculation formula into the instrument, the calculation of HbA1c ratio from total hemoglobin is done by the instrument automatically. Please refer to the instrument manual.

Dependent on selected standardization enter the following formula:

IFCC

Values in mmol/mol according to 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

For calibration the DiaSys TruCal HbA1c net calibrator is recommended. The assigned values of TruCal HbA1c net have been made traceable to the approved IFCC reference method [1]. For internal quality control, DiaSys TruLab HbA1c net controls should be assayed. 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
On-board stability	6 weeks
Calibration stability	6 weeks

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

Specificity/Interferences

According to CLSI protocol EP7-A2 a study on interferences was conducted.

IFCC

For each interfering substance three samples with different hemoglobin and HbA1c values have been tested; a low level sample within a hemoglobin range of 8 – 10 g/dL and a HbA1c range within 28 – 35 mmol/mol; a medium level sample within a hemoglobin range of 11 – 15 g/dL and a HbA1c range within 28 – 35 mmol/mol; a high level sample within a hemoglobin range of 11 – 15 g/dL and a HbA1c range > 60 mmol/mol.

DCCT/NGSP

For each interfering substance three samples with different hemoglobin and HbA1c values have been tested; a low level sample within a hemoglobin range of 9 – 10 g/dL and a HbA1c range within 4.7 – 5.4%; a medium level sample within a hemoglobin range of 10 – 15 g/dL and a HbA1c range within 4.7 – 5.4%; a high level sample within a hemoglobin range of 10 - 15 g/dL and a HbA1c range > 7.65%.

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

Interfering substance	**Interferences < 10% in serum with hematocrit correction**
Ascorbate	up to 50 mg/dL
Bilirubin (conjugated and unconjugated)	up to 10 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 1000 mg/dL
N-acetylcysteine (NAC)	up to 2000 mg/L
Urea	up to 300 mg/dL
Uric acid	up to 20 mg/dL
Alcoholism and ingestion of large doses of aspirin may lead to implausible results. For further information on interfering substances refer to Young DS [10].	

Hemoglobin variants can 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

Imprecision

Values according to IFCC

Within-run precision n = 20	Mean [mmol/mol]	SD [mmol/mol]	CV [%]
Sample 1	32.7	0.309	0.947
Sample 2	33.2	0.207	0.623
Sample 3	63.7	0.308	0.483

Total precision CLSI n = 80	Mean [mmol/mol]	SD [mmol/mol]	CV [%]
Sample 1	32.1	0.522	1.63
Sample 2	33.6	0.433	1.29
Sample 3	67.6	0.824	1.22

Method comparison (n=100)	
Test x	Competitor enzymatic HbA1c assay
Test y	DiaSys HbA1c net FS
Slope	0.983
Intercept	0.772 mmol/mol
Coefficient of correlation	0.9945

Method comparison (n=100)	
Test x	HPLC assay
Test y	DiaSys HbA1c net FS
Slope	0.996
Intercept	-0.0153 mmol/mol
Coefficient of correlation	0.9931

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

1. 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.
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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: 1756-8.
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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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Hemoglobin TWIN test Application for HbA1c net FS

Reagent code 10 334

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.

Sub Param. : 2 (Up button)

Analytical Conditions	
R1 volume	90
R2e volume	0
R2 volume	30
R1 diluent vol	0
R2e diluent vol	0
R2 diluent vol	0
Sample vol (S)	15
Sample vol (U)	15
Reagent 1 mix	strong
Reagent 2e mix	weak
Reagent 2 mix	weak
Reaction time	10

Sub-analy. Conditions	
Name	Hbn
Digits	2
Unit	g/dL
M-wave. L.	571
S-wave. L.	805
Analy.mthd.	EPA
Calc.mthd.	MSTD
Qualit. judge	Not do

Calculation Method Setting	
M-DET.P.I	0
M-DET.P.m	17
M-DET.P.n	18
Check D.P.	0
Limit value	0.003
Variance	10
S-DET.P.p	0
S-DET.P.r	0

Prozone (MULTI-STD Setting only)	
Prozone form	None
Prozone limit	9.999
Prozone judge	Upper limit
Judge limit	9.999
M-DET.P.m	0
M-DET.P.n	0
S-DET.P.p	0
S-DET.P.r	0

Reac. Type	
Reac. Type	Inc.

Reaction Rate Method	
Cycle	2
Factor	2.0
E2 corre	Not do
Blank (u)	9.999
Blank (d)	-9.999
Sample (u)	9.999
Sample (d)	-9.999

Endpoint Method	
Re.absorb (u)	9.999
Re.absorb (d)	-9.999

Analysis item condition setting (M) [Subwindow button]		
Sample Type *	**Serum**	**Urine**
Reac. sample vol.	15	15
Diluent method	With dilution	With dilution
Undil. sample vol.	5	5
Diluent volume	100	100
Diluent position	**	**

*Has to be defined for controls and Blank as well

**Position number must be between 1 and 45 respectively 1 and 50 (depends on rotor)

Standards setting					
FV	#	BLK H	9.999	STD H	9.999
		BLK L	-9.999	STD L	-9.999

Multi-STD setting (subwindow button)

Formula: Linear correction | **Blank:** Any value | **Axis conv. :** No convert. | **Points:** 2

FV		**Reac. smp. vol.**	**Dil. method**	**Undiluted. smp. vol.**	**Diluent volume**	**Diluent position**	**STD-H**	**STD-L**
BLK/0	#	15	With dilution	1.6	100	**	9.999	-9.999
1	#	15	With dilution	5.0	100	**	9.999	-9.999
2								
3								
4								
5								

#entered by user

For adjustment and maintenance of the sample probe, please contact your local distributor/service technician.

Individual results for hemoglobin and HbA1c in g/dL must not be used for diagnostic purposes.

HbA1c TWIN test Application for HbA1c net FS

Reagent code 10 334

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.

Sub Param. : 1

Analytical Conditions	
R1 volume	90
R2e volume	0
R2 volume	30
R1 diluent vol	0
R2e diluent vol	0
R2 diluent vol	0
Sample vol (S)	15
Sample vol (U)	15
Reagent 1 mix	strong
Reagent 2e mix	weak
Reagent 2 mix	weak
Reaction time	10

Sub-analy. Conditions	
Name	HbA1cn
Digits	3
Unit	g/dL
M-wave. L.	658
S-wave. L.	805
Analy.mthd.	EPA
Calc.mthd.	MSTD
Qualit. judge	Not do

Calculation Method Setting	
M-DET.P.l	0
M-DET.P.m	41
M-DET.P.n	42
Check D.P.	0
Limit value	0.003
Variance	10.0
S-DET.P.p	22
S-DET.P.r	23

Prozone (MULTI-STD Setting only)	
Prozone form	None
Prozone limit	9.999
Prozone judge	Upper limit
Judge limit	9.999
M-DET.P.m	0
M-DET.P.n	0
S-DET.P.p	0
S-DET.P.r	0

Reac. Type	
Reac. Type	Inc.

Reaction Rate Method	
Cycle	2
Factor	2.0
E2 corre	Not do
Blank (u)	9.999
Blank (d)	-9.999
Sample (u)	9.999
Sample (d)	-9.999

Endpoint Method	
Re.absorb (u)	9.999
Re.absorb (d)	-9.999

Analysis test condition setting (M) [Subwindow button]		
Sample Type *	**Serum**	**Urine**
Reac. sample vol.	15	15
Diluent method	With dilution	With dilution
Undil. sample vol.	5	5
Diluent volume	100	100
Diluent position	**	**

* Has to be defined for controls and Blank as well

**Position number must be between 1 and 45 respectively 1 and 50 (depends on rotor)

Standards setting					
FV	#	BLK H	9.999	STD H	9.999
		BLK L	-9.999	STD L	-9.999

Multi-STD setting (subwindow button)

Formula: Linear correction | **Blank:** Any value | **Axis conv. :** No convert. | **Points:** 2

FV		**Reac. smp. vol.**	**Dil. method**	**Undiluted. smp. vol.**	**Diluent volume**	**Diluent position**	**STD-H**	**STD-L**
BLK/0	#	15	With dilution	1.6	100	**	9.999	-9.999
1	#	15	With dilution	5.0	100	**	9.999	-9.999
2								
3								
4								
5								

#entered by user

For adjustment and maintenance of the sample probe, please contact your local distributor/service technician.

Individual results for hemoglobin and HbA1c in g/dL must not be used for diagnostic purposes.