

# HbA1c<sup>®</sup>net FS\*

## Order Information

Cat. No. 1 3348 99 10 930 Kit size R1 3 x 18 mL + R2 3 x 6 mL

Kits for use in conjunction with DiaSys CE applications.

## Intended Use

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

## Summary

Hemoglobin A1c (HbA1c) is glycosylated hemoglobin which is formed by the non-enzymatic attachment of glucose to native hemoglobin. The amount of HbA1c is dependent on the total quantity of hemoglobin. For this reason, HbA1c value is expressed as the ratio of glycosylated hemoglobin to total hemoglobin [1,2]. The rate of glycosylation is directly proportional to the blood glucose level. As the average lifespan of erythrocytes is around 120 days, the HbA1c value reflects the glycemic status over this period [1]. Determination of HbA1c is recommended for different purposes across age groups. For adolescents and adults, it can be used for screening the risk of diabetes and to diagnose a manifest diabetes, especially type 2 diabetes [1,3]. In addition, HbA1c is used to monitor long-term glycemic status in diabetics to track the success of the respective therapy, as clinical studies have shown that lowering HbA1c might help to prevent or to delay late diabetic complications [1,2]. In children, however, determination of HbA1c is only recommended for screening for increased diabetes risk [4].

## Method

Hemoglobin: Photometric test  
HbA1c: Colorimetric, enzymatic method

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 570 nm after addition of reagent R1 and is proportional to the total hemoglobin concentration in the sample.

### HbA1c measurement [5]

After addition of R2, fructosylated dipeptides from the N-terminal part of the hemoglobin  $\beta$ -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 660 nm. The absorbance increase is proportional to the HbA1c concentration.

## Standardization

The assay is standardized according to the approved IFCC reference method [6].

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

$$HbA1c (IFCC^a) = (HbA1c (NGSP^b) - 2.15) / 0.0915$$

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

a: IFCC values in mmol/mol

b: NGSP values in %

IFCC: International Federation of Clinical Chemistry [6-8]

DCCT: Diabetes Control and Complications Trial [9]

NGSP: National Glycohemoglobin Standardization Program [10]

## HbA1c and Average Glucose Concentrations

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 [11]:

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

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

a: HbA1c values in mmol/mol IFCC

Standardization according to NGSP:

$$\text{Average glucose conc. [mg/dL]} = 28.7 \times HbA1c^b - 46.7$$

$$\text{Average glucose conc. [mmol/L]} = 1.59 \times HbA1c^b - 2.59$$

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	100 mmol/L
	FPOX	$\geq 0.5$ kU/L
	Ethylene glycol derivative	< 10%
R2:	Buffer	20 mmol/L
	Protease	$\geq 500$ kU/L
	Chromogen	$\geq 0.05$ mmol/L
	Ethylene glycol derivative	< 10%

## Storage and Stability

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C and contamination is avoided. Do not freeze and protect from light.

The open-vial stability of the reagent is 12 months until expiry date.

## Warnings and Precautions

1. Components contained in HbA1c net FS are classified according to EC regulation 1272/2008 (CLP) as follows:



⚠ Reagent 2: Warning. H400 Very toxic to aquatic life. P273 Avoid release to the environment. P391 Collect spillage. P501 Dispose of contents/container to hazardous or special waste collection point.

2. The reagents contain material of biological origin. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
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. Measurement of HbA1c is not appropriate for diagnosis of gestational diabetes [12].
5. 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 [1].
6. As HbA1c represents the stable coupling of glucose at the N-terminal end of the hemoglobin A1  $\beta$ -chain, glycosylated Hb variants without  $\beta$ -chains cannot be determined with this test. Determination of total hemoglobin includes all Hb variants; therefore, samples with high concentrations of Hb variants without  $\beta$ -chains may show falsely low HbA1 concentrations.

7. In very rare cases, samples of patients with gammopathy might give falsified results [13].
8. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
9. In case of product malfunction or altered appearance that could affect the performance, contact the manufacturer.
10. Any serious incident related to the product must be reported to the manufacturer and the competent authority of the Member State where the user and/or patient is located.
11. Please refer to the safety data sheets (SDS) 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.
12. For professional use only.

## Waste Management

Refer to local legal requirements for chemical disposal regulations as stated in the relevant SDS to determine the safe disposal.

Warning: Handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

## Reagent Preparation

The reagents are ready to use.

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

## Materials Required

General laboratory equipment

## Specimen

Human EDTA whole blood

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

Only use suitable tubes or collection containers for specimen collection and preparation.

When using primary tubes, follow the manufacturer's instructions.

Stability [14]:

Whole blood	1 week	at	2 – 8°C
Hemolysate	1 hour	at	15 – 25°C

Discard contaminated specimens.

## Sample Preparation

For sample preparation the DiaSys HbA1c net Hemolyzing Solution Cat. No. 1 4590 99 10 113 is required.

Calibrators, controls and samples have to be hemolyzed before use. Hemolysates have to be processed within 1 hour after production. Processing in batch mode is recommended.

Please refer to subsequent pipetting scheme for manual hemolysis:

	Preparation			
	Calibrator Level 1	Calibrator Level 2	Control	Sample
TruCal HbA1c net Level 1	16 µL	-	-	-
TruCal HbA1c net Level 2	-	50 µL	-	-
TruLab HbA1c net Level 1 and Level 2 /Sample	-	-	50 µL	50 µL
Add				
HbA1c net Hemolyzing solution	1000 µL	1000 µL	1000 µL	1000 µL
Mix and allow standing for 1 minute. Hemolysis is completed after 1 minute. A slight turbidity remains due to the composition of the hemolyzing solution.				

## Assay Procedure

**Basic settings for BioMajesty® JCA-BM6010/C (TWIN test setting)**

### Hemoglobin determination

<b>Wavelength</b>	571/805 nm
<b>Temperature</b>	37°C
<b>Measurement</b>	Endpoint
<b>Sample/Calibrator</b>	15 µL
<b>Reagent 1</b>	90 µL
<b>Reagent 2</b>	-
<b>Absorbance 1</b>	Cycle 17/18 (231 s/244 s)
<b>Absorbance 2</b>	-
<b>Calibration</b>	Linear

### HbA1c determination

<b>Wavelength</b>	658/805 nm
<b>Temperature</b>	37°C
<b>Measurement</b>	Endpoint
<b>Sample/Calibrator</b>	15 µL
<b>Reagent 1</b>	90 µL
<b>Reagent 2</b>	30 µL
<b>Addition reagent 2</b>	Cycle 19 (286 s)
<b>Absorbance 1</b>	Cycle 22/23 (327 s/340 s)
<b>Absorbance 2</b>	Cycle 41/42 (586 s/600 s)
<b>Calibration</b>	Linear

## 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.

Depending on the standardization selected, 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

DiaSys TruCal HbA1c net is recommended for calibration. Calibrator values have been made traceable to the approved IFCC reference method [6]. Use DiaSys TruLab HbA1c net Level 1 and Level 2 for internal quality control. All target values of the controls are traceable to DiaSys reagent/calibrator system. Quality control must be performed after calibration. Control intervals and limits have to be adapted to the individual requirements of each laboratory. Results must be within the defined ranges. Follow the relevant legal requirements and guidelines. 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

### Data evaluated on BioMajesty® JCA-BM6010/C

Measuring range from 20 mmol/mol up to 150 mmol/mol according to IFCC (from 4% up to 16% according to DCCT/NGSP). Linearity IFCC < 30 mmol/mol is given with ± 1.5 mmol/mol, linearity between 30 mmol/mol up to 100 mmol/mol within ± 5%, linearity > 100 mmol/mol within ± 7%. The assay is applicable for hemoglobin concentrations in blood from 6 g/dL to 30 g/dL (from 3.73 mmol/L to 18.6 mmol/L). Linearity is given within ± 5%.

Limit of detection**	HbA1c: 0.3 g/dL Hemoglobin: 6 g/dL
Limit of quantitation**	HbA1c: 0.3 g/dL Hemoglobin: 6 g/dL

Interference by	Interferences ≤ 10% in serum with hematocrit correction up to	Analyte concentration [mmol/mol]
<b>Ascorbic acid</b>	50 mg/dL	31.7
	50 mg/dL	67.7
<b>Bilirubin</b> (conjugated)	10 mg/dL	34.4
	10 mg/dL	70.5
<b>Bilirubin</b> (unconjugated)	10 mg/dL	32.4
	10 mg/dL	70.9
<b>Glucose</b>	1000 mg/dL	34.9
	1000 mg/dL	60.8
<b>Hemoglobin</b> (acetylated)	10 mmol/L	34.6
	10 mmol/L	70.6
<b>Hemoglobin</b> (carbamyated)	10 mmol/L	34.8
	10 mmol/L	70.0
<b>Lipemia</b> (triglycerides)	1000 mg/dL	31.5
	1000 mg/dL	67.4
<b>N-acetylcysteine</b> (NAC)	2000 mg/L	32.3
	2000 mg/L	70.6
<b>Urea</b>	300 mg/dL	31.2
	300 mg/dL	66.9
<b>Uric acid</b>	20 mg/dL	34.1
	20 mg/dL	69.8

For further information on interfering substances, refer to the literature [1,15-17].

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

Repeatability (n=20)	Sample 1	Sample 2	Sample 3
Mean [mmol/mol]	32.7	33.2	63.7
CV [%]	0.947	0.623	0.483
Within-laboratory (n=80)	Sample 1	Sample 2	Sample 3
Mean [mmol/mol]	32.1	33.6	67.6
CV [%]	1.63	1.29	1.22

### Method comparison (n=100)

Test x	HPLC Arkray HA-8160 V7.41 (Arkray HA-8160 V7.41)
Test y	DiaSys HbA1c net FS (BioMajesty® JCA-BM6010/C)
Slope	0.996
Intercept	-0.015 mmol/mol
Coefficient of correlation	0.993

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

## Reference Range

**Suggested target values for HbA1c [18]:**

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

**HbA1c cut point value for diagnosis of diabetes mellitus [2]:**

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

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

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

## Literature

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