

# HbA1c FS\*

Diagnostic reagent for quantitative in vitro determination of hemoglobin A1c (HbA1c) in whole blood on photometric systems

## Order Information

Cat. No.	Kit size
1 3348 99 10 930	R1 3 x 18 mL + R2 3 x 6 mL
1 4590 99 10 113	1 x 500 mL HbA1c net Hemolyzing Solution
1 3350 99 10 044	2 x 0.3 mL TruCal HbA1c net

## Summary [1,2,3,11,14]

Hemoglobin A1c (HbA1c) is a glycosylated hemoglobin which is formed by the non-enzymatic reaction of glucose with native hemoglobin. This process runs continuously throughout the circulatory life of erythrocytes (average life time 100 – 120 days). The rate of glycation is directly proportional to the concentration of glucose in blood. The blood level of HbA1c represents the average blood glucose level over the last 3 months. Therefore, HbA1c is suitable for retrospective long-term monitoring of blood glucose concentration in diabetics. Clinical studies have shown that lowering of HbA1c level can help to prevent or delay the incidence of late diabetic complications. Besides, HbA1c testing can be used for diagnosis of diabetes mellitus.

As the amount of HbA1c also depends on the total quantity of hemoglobin, the HbA1c value is indicated as ratio of the total hemoglobin concentration.

## 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 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  $\beta$ -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 IFCC [4] and DCCT/NGSP [7] 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:

$$\begin{aligned} \text{HbA1c (IFCC}^b) &= (\text{HbA1c (NGSP}^a) - 2.15) / 0.0915 \\ \text{HbA1c (NGSP}^a) &= 0.0915 \times \text{HbA1c (IFCC}^b) + 2.15 \end{aligned}$$

a: NGSP values in %

b: IFCC values in mmol/mol

IFCC: International Federation of Clinical Chemistry [4,5,10]

DCCT: Diabetes Control and Complications Trial [6]

NGSP: National Glycohemoglobin Standardization Program [7]

## HbA1c and Average Glucose Concentrations [11]

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 [11]):

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

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

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 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	$\geq 0.5$ 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 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!

### 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!

### Warnings and Precautions

- The reagents contain material of biological origin. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
- 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.
- 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.
- In very rare cases, samples of patients with gammopathy might give falsified results [15].
- N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
- Please refer to the safety data sheets and take the necessary precautions for use of laboratory reagents. For diagnostic purposes, results should always be assessed with the patient's medical history, clinical examinations and other findings.
- For professional use only!

### Waste Management

Please refer to local legal requirements.

### Materials Required but not Provided

General laboratory equipment

## 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 [8]:

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

Discard contaminated specimens.

## Sample Preparation

DiaSys HbA1c net Hemolyzing Solution is required for sample preparation. 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

*Application sheets for automated systems are available on request. Please refer to your distributor.*

**Basic parameters for Hitachi 917 with TWIN application and manual calibrator/control/sample hemolization**

### Hemoglobin determination

<b>Wavelength (main/sub)</b>	570/800 nm (bi-chromatic)
<b>Temperature</b>	37°C
<b>Measurement</b>	TWIN test/3-point
<b>Sample/Calibrator</b>	30 µL
<b>Reagent 1</b>	180 µL
<b>Reagent 2</b>	60 µL
<b>Addition Reagent 2</b>	Cycle 15
<b>Absorbance</b>	Cycle 15
<b>Calibration</b>	linear

### HbA1c determination

<b>Wavelength (main/sub)</b>	660/800 nm (bi-chromatic)
<b>Temperature</b>	37°C
<b>Measurement</b>	TWIN test/3-point
<b>Sample/Calibrator</b>	30 µL
<b>Reagent 1</b>	180 µL
<b>Reagent 2</b>	60 µL
<b>Addition Reagent 2</b>	Cycle 15
<b>Absorbance 1</b>	Cycle 18
<b>Absorbance 2</b>	Cycle 34
<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.

Stability of calibration: 6 weeks

## 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 calibrator is recommended for calibration. The assigned values of TruCal HbA1c net have been made traceable to the approved IFCC reference method [4]. DiaSys TruLab HbA1c net controls should be assayed for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
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

The assay has got a 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).

### 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
<b>Ascorbate</b>	up to 50 mg/dL
<b>Bilirubin</b> (conjugated and unconjugated)	up to 10 mg/dL
<b>Glucose</b>	up to 1000 mg/dL
<b>Hemoglobin, acetylated</b>	up to 10 mmol/L
<b>Hemoglobin, carbamylated</b>	up to 10 mmol/L
<b>Lipemia</b> (triglycerides) at < 11 g/dL hemoglobin	up to 400 mg/dL
<b>Lipemia</b> (triglycerides) at > 11 g/dL hemoglobin	up to 750 mg/dL
<b>N-acetylcysteine (NAC)</b>	up to 2000 mg/L
<b>Urea</b>	up to 300 mg/dL
<b>Uric acid</b>	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 [13].	

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

### Sensitivity/Limit of Detection

HbA1c: 0.2 g/dL

Hemoglobin: 1.5 g/dL

## Imprecision

Values according to IFCC (Hitachi 917)

Within-run precision n = 20	Mean [mmol/mol]	SD [mmol/mol]	CV [%]
Sample 1	29.5	0.556	1.88
Sample 2	32.9	0.197	0.598
Sample 3	63.5	0.447	0.703

Total precision CLSI n = 80	Mean [mmol/mol]	SD [mmol/mol]	CV [%]
Sample 1	26.0	1.01	3.88
Sample 2	32.5	1.23	3.78
Sample 3	66.2	1.23	1.86

## Method Comparison

A comparison of DiaSys HbA1c net FS (y) to an immunoturbidimetric assay (x) using 60 samples gave following results (IFCC values):

$$y = 1.047 x - 0.782 \text{ mmol/mol}; r = 0.982$$

A comparison of DiaSys HbA1c net FS (y) to a HPLC assay (x) using 100 samples gave following results (IFCC values):

$$y = 1.031 x + 0.441 \text{ mmol/mol}; r = 0.989$$

## Reference Range

**Suggested target values for HbA1c [9]:**

	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):  $\geq 6.5\%$  (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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