Instructions for Use
For *in vitro* diagnostic use

HbA1c Kit

HbA1c Kit

Anvisa 80115310145

ORDER INFORMATION

Cat. No.	Kit size
4190045K	R1: 2 x 15 mL + R2: 1 x 10 mL + R3: 1 x 5 mL
4190045MK	R1: 1 x 30 mL + R2: 1 x 10 mL + R3: 1 x 5 mL

INTENDED USE

Diagnostic reagent for quantitative *in vitro* determination of Hemoglobin A1c levels in whole blood on photometric systems.

SUMMARY [1, 2, 3]

Hemoglobin A1c (HbA1c) is a glycated hemoglobin which is formed by the non-enzymatic reaction of glucose with native hemoglobin. This process runs continuously throughout the circulatory life of the red cell (average lifetime 100 - 120 days). The rate of glycation is directly proportional to the concentration of glucose in the blood. The blood level of HbA1c represents the average blood glucose level over the preceding 6 to 8 weeks (due to the kinetics of erythrocyte turnover this period is more affected by the blood glucose level than the preceding weeks). Therefore, HbA1c is suitable for retrospective long-term monitoring of blood glucose concentration in individuals with diabetes mellitus. Clinical studies have shown that lowering of HbA1c level can help to prevent or delay the incidence of late diabetic complications. Besides, HbA1c testing may be used for diagnosis of diabetes mellitus.
As the amount of HbA1c also depends on the total quantity of hemoglobin the reported HbA1c value is indicated as a percentage of the total hemoglobin concentration.
Falsely low values (low HbA1c despite high blood glucose) may occur in people with conditions with shortened red blood cell survival (hemolytic diseases) or significant recent blood loss (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.

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.

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	

STANDARDIZATION [4]

The assay is standardized according to the approved IFCC reference method. Calibration according to DCCT/NGSP is also possible. The values of corresponding calibrators are listed at the instructions for use of the liquid calibrators TopKal HbA1c.
NGSP and IFCC values show a linear relationship and can therefore 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 [4, 5, 6]
DCCT: Diabetes Control and Complications Trial [7]
NGSP: National Glycohemoglobin Standardization Program [8]

EQUATION FOR CONVERTING IFCC PERCENTAGE VALUES TO MMOL/MOL:

[mmol/mol IFCC] = 10 x [% IFCC]

HBA1C AND AVERAGE GLUCOSE CONCENTRATIONS [9]

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:
Average glucose concentration [mg/dL] = $2.63 \times HbA1c^a + 15.01$
Average glucose concentration [mmol/L] = $0.146 \times HbA1c^a + 0.829$
a: HbA1c values in mmol/mol IFCC

Standardization according to NGSP:
Average glucose concentration [mg/dL] = $28.7 \times HbA1c^b - 46.7$
Average glucose concentration [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.

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 [10].
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. Immediately after HbA1c measurement cleaning of cuvettes is necessary. Use the alkaline cuvette washing solution which is recommended by the analyzer manufacturer.
6. For professional use only!

STORAGE AND STABILITY

The reagents are stable up to the end of the label-indicated month of expiry, if stored at 2 – 8°C and contamination is avoided. Do not freeze the reagents and protect them from light.

WASTE MANAGEMENT

Follow the requirements of the current guidelines about technical regulation for the management of healthcare service waste, as well as other equivalent biosafety practices.

REAGENT PREPARATION

Reagent 1 is ready to use. Reagent 2 and reagent 3 must be premixed before use. Transfer 5 mL of R3 into one bottle R2 and mix well immediately.
Stability of premixed R2/R3: One month stored at 2 – 8°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. NaCl Solution (9 g/L)
2. General laboratory equipment.

SPECIMEN

Whole blood collected with EDTA.
Discard contaminated specimens.
Please collect whole blood by standard venipuncture and fill the blood collection tube according to manufacturer specifications.
For sample preparation the Hemolyzing Solution "Solução Hemolisante" is required.

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 [11]

Whole blood	1 week	At	2-8°C
Hemolysate	10 hours	at	15-25°C
Hemolysate	10 days	At	2-8°C

ASSAY PROCEDURE

Application sheets for automated systems are available on request or at our site www.kovalent.com.br

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Wavelength:	660 nm
Optical path:	1 cm
Temperature:	37ºC
Measurement:	Against air

2-component system - premixed R2/R3

Sample or calibrator	15 µL
Reagent 1	600 µL
Mix, incubate for 5 min., then add:	
Reagent 2/3	300 µL
Mix, read absorbance (A1) after exactly 1 min and read absorbance (A2) after a total of exactly 5 min.	

∆A = (A2-A1) sample or calibrator

CALIBRATORS AND CONTROLS

TopKal HbA1c from Kovalent 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 TopKon HbA1c nível 1 and TopKon HbA1c Nível 2 from Kovalent for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

WARRANTY

These instructions for use should be read carefully before using the product and the information contained therein should be strictly adhered to. The reliability of the test results cannot be guaranteed if the instructions are not followed.

PERFORMANCE CHARACTERISTICS

Measuring Range

The test has been developed to determine concentrations of HbA1c within a measuring range from 30 – 150 mmol/mol according to IFCC (4.9 – 16% according to NGSP), at least up to the concentration of the highest calibrator. The assay is applicable for hemoglobin concentrations in blood from 6.6 to 26 g/dL.

Interferences

The study on interferences was conducted according to CLSI protocols.

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 for the 2-component system.

Interfering substance	Interferences <7% DCCT/NGSP and <10% IFCC
Ascorbate	up to 60 mg/dL
Bilirrubin (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 (tryglicerides)	up to 2000 mg/dL
N-acetylcysteine (NAC)	up to 1000 mg/dL
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 Young DS [12].	

Hemoglobin variants [11]:

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.

Sensitivity / Limit of Detection

The limit of detection (LOQ) is 30 mmol/mol HbA1c according to IFCC (4.9% HbA1c according to DCCT/NGSP).

PRECISION (HITACHI 917, 2-COMPONENT SYSTEM)

Values according to IFCC:

Within-run precision n = 20	Mean [mmol/mol]	SD [mmol/mol]	CV [%]
Sample 1	36.4	0.572	1.57
Sample 2	60.0	0.522	0.869
Sample 3	87.6	1.01	1.15

Between day precision n = 20	Mean [mmol/mol]	SD [mmol/mol]	CV [%]
Sample 1	34.9	0.678	1.94
Sample 2	53.9	0.953	1.77
Sample 3	86.6	0.920	1.06

METHOD COMPARISON

A comparison of Kovalent's HbA1c (y) to a commercially available immunoturbidimetric assay (x) using 88 samples gave the following results (IFCC values):
y = 1.01 x – 1.10 mmol/mol; r = 0.997

A comparison of Kovalent's HbA1c (y) to a HPLC assay (x) using 100 samples gave following results (IFCC values):
y = 1.01 x – 0.437 mmol/mol; r = 0.997

REFERENCE VALUES

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

HbA1c cut point value for diagnosis of diabetes mellitus [3]:
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.

The reference values should be established or verified by each laboratory, based on an adequate population of non-diabetics.

Suggested target values for HbA1c [13]:

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

LITERATURE

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4. 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.
5. Hoelzel W, Weykamp C et al. IFCC Reference System for Measurement of Hemoglobin A1c in Human Blood and the National Standardization Schemes in the United States, Japan, and Sweden: A Method-Comparison Study. Clin Chem 2004; 50 (1): 166-74.
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8. 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.
9. Sacks DB. Translating Hemoglobin A1c into Average Blood Glucose: Implications for Clinical Chemistry. Clinical Chemistry 2008; 54: 1756-8.
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11. Data on file at DiaSys Diagnostic Systems GmbH.
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CONSUMER INFORMATION

Symbols used:

	Manufacturer
	Temperature limit
	In vitro diagnostic device
	Caution
	Operating instructions
	Recycling material
	Do not discard directly into the environment
	Batch code
	Date of manufacture
	Use by date
	Biological hazards
	Highly toxic
	Corrosive
	Harmful

Manufacturer:

Kovalent do Brasil Ltda.
Rua Cristóvão Sardinha, 110 – Jd. Bom Retiro
São Gonçalo – RJ – CEP 24722-414
www.kovalent.com.br
CNPJ: 04.842.199/0001-56

Kit sizes variations on demand:

Anvisa No.	Kit size
80115310145	R1: 3 x 20 mL + R2: 2 x 10 mL + R3: 1 x 10 mL

Customer Service: sac@kovalent.com.br - (21) 3907-2534 / 0800 015 1414

Expiration date and Lot no.: See label