16.9	13.43	—	18.7	14.94	—
17.0	13.51	—	18.8	15.02	—
17.1	13.60	—	18.9	15.11	—
17.2	13.68	—	19.0	15.19	—
17.3	13.77	—	19.1	15.27	—
17.4	13.85	—	19.2	15.36	—
17.5	13.93	—	19.3	15.44	—
17.6	14.02	—	19.4	15.53	—
17.7	14.10	—	19.5	15.61	—
17.8	14.18	—	19.6	15.69	—
17.9	14.27	—	19.7	15.78	—
18.0	14.35	—	19.8	15.86	—
18.1	14.44	—	19.9	15.94	—
18.2	14.52	—	20.0	16.03	—
18.3	14.60	—			
18.4	14.69	—			
18.5	14.77	—			
18.6	14.85	—			

In the test study done by Nathan D.M. et.al. They calculated the Mean Blood Glucose concentration from the value of HbA1c% measured with the equation MBG in mg/dl = 33.3 x HbA1c value - 86.
These values are linear in the range of 6.5 - 13% of HbA1c values.

Mfd. for:

Singapore Biosciences PTE Ltd.

11 Yishun Street 51, # 04-23, The Criterion, Singapore 767971.

EC REP

CMC Medical Devices & Drugs S.L.,
C/ Horacio Lengo No. 18, CP 29006, Malaga, Spain.

GHB1(SBio)/0420/VER-02

SBio GLYCOSYLATED HAEMOGLOBIN KIT

(Ion Exchange Resin Method)

(For invitro diagnostic use only)

REF	90540010	90540025
Σ	10 Tests	25 Tests

CE

8°C 2°C Store at 2-8°C	Manufacturer	This way up	Ion Exchange Resin — Ion Exchange Resin Method
Use by (Last day of stated month)	Consult Instructions for use	L1 Ion Exchange Resin	L2 Lysing Reagent
Date of Manufacture	REF Catalogue Number	A Resin Separators	C Control (10 % GHb)
LOT Batch Number	IVD *In vitro* Diagnostic Medical Device	Σ Contains sufficient for <n> tests	EC REP Authorised Representative in the European Community

INTENDED USE

Glycosylated Hemoglobin Kit is used for the determination of glycohemoglobin in blood.

PRINCIPLE OF THE TEST

Glycosylated hemoglobin (GHb) has been defined operationally as the fast fraction hemoglobins HbA1 (Hb A1a, A1b, A1c) which elute first during column chromatography. The non - glycosylated hemoglobin, which consists of the bulk of hemoglobin, has been designated HbAo.

A hemolysed preparation of whole blood is mixed continuously for 5 minutes with a weakly binding cation-exchange resin. The labile fraction is eliminated during the hemolysate preparation and during the binding. During this mixing, HbAo binds to the ion exchange resin leaving GHb free in the supernatant. After the mixing period, a filter separator is used to remove the resin from the supernatant. The percent glycosylated hemoglobin is determined by measuring absorbances of the glycosylated hemoglobin (GHb) fraction and the total hemoglobin fraction (THb) fraction. The ratio of the absorbances of the Glycosylated hemoglobin & the Total hemoglobin fraction of the Control and the Test is used to calculate the percent Glycosylated hemoglobin of the sample.

CLINICAL SIGNIFICANCE

Glycosylated (or glycated) hemoglobin is a form of hemoglobin used primarily to identify the plasma glucose concentration over prolonged periods of time. In the normal 120-day life span of the red blood cell, glucose molecules join hemoglobin, forming glycated hemoglobin. In individuals with poorly controlled diabetes, increases in the quantities of these glycated hemoglobins are noted. Once a hemoglobin molecule is glycated, it remains that way. A buildup of glycated hemoglobin within the red cell reflects the average level of glucose to which the cell has been exposed during its life cycle. Measuring glycated hemoglobin assesses the effectiveness of therapy by monitoring long-term serum glucose regulation.

PRESENTATION	10 Tests	25 Tests
L1 : Ion Exchange Resin (Predispensed Tubes)	10 x 3 ml	25 x 3 ml
L2 : Lysing Reagent	5 ml	12.5 ml
C : Control (10% GHb)	1 ml	1 ml
A : Resin Separators	10 Nos.	25 Nos.

COMPOSITION

Goods Buffer 50mM; Pre weighed amount of resin equilibrated with Goods Buffer pH 6.8; Non Stabilizer, Detergents and Preservatives.

STORAGE / STABILITY

Contents are stable at 2-8^0 C till the expiry mentioned on the labels. Do not freeze. The Resin separators can be removed on opening the kit and stored at R.T.

SAMPLE REQUIRED

Whole blood is required. Preferably fresh & collected in EDTA.

MATERIAL REQUIRED BUT NOT PROVIDED

General laboratory instrumentation like Spectrophotometer/Analyzer, Thermostatic Cuvette holder, Cuvettes, Micropipettes, Test tubes, Waterbath, Stopwatch/Timer.

REAGENT PREPARATION

The Ion Exchange Resin tubes & the Lysing Reagent are ready to use. Reconstitute the Control with 1 ml of distilled water. Allow to stand for 10 mins with occasional mixing. The reconstituted control is stable for at least 7 days when stored at 2-8^{0}C tightly sealed, and at least 4 weeks when stored at -20^{0}C. Do not thaw and refreeze.

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.

This product requires the handling of human specimen. It is recommended that all human sourced material are considered potentially hazardous and are handled in accordance with the OSHA standard on blood borne pathogens.

Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

Handle specimen, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

TEST PROCEDURE

Wavelength / filter : 415 nm (Hg 405 nm)
Temperature : R.T.
Light path : 1 cm

A. Hemolysate Preparation

1. Dispense 0.5 ml Lysing Reagent into tubes labeled as Control (C) & Test (T).
2. Add 0.1ml of the reconstituted control & well-mixed blood sample into the appropriately labeled tubes. Mix until complete lysis is evident.
3. Allow to stand for 5 minutes.

B. Glycosylated hemoglobin (GHb) Separation

1. Remove cap from the Ion-Exchange Resin tubes and label as Control & Test.
2. Add 0.1 ml of the hemolysate from Step A into the appropriately labeled Ion Exchange Resin tubes.
3. Insert a resin Separator into each tube so that the rubber sleeve is approximately 1 cm above the liquid level of the resin suspension.
4. Mix the tubes on a rocker, rotator or a vortex mixer continuously for 5 minutes.
5. Allow the resin to settle, then push the resin separator into the tubes until the resin is firmly packed.
6. Pour or aspirate each supernatant directly into a cuvette and measure each absorbance against distilled water.

C. Total Hemoglobin (THb) fraction

1. Dispense 5.0 ml of distilled water into tubes labeled as Control & Test.
2. Add to it 0.02 ml of hemolysate from Step A into the appropriately labeled tube.
3. Mix well.
4. Read each absorbance against distilled water.

CALCULATIONS

$$\text{Ratio of Control } (R_c) = \frac{\text{Abs. Control GHb}}{\text{Abs. Control THb}}$$

$$\text{Ratio of Test } (R_T) = \frac{\text{Abs. Test GHb}}{\text{Abs. Test THb}}$$

$$\text{GHb in \%} = \frac{\text{Ratio of Test } (R_T)}{\text{Ratio of Control } (R_c)} \times 10 \text{ (Value of Control)}$$

QUALITY CONTROL

The following process is recommended for QC during the assay of GHb.
*Define and establish acceptable range for your laboratory.

1. Two levels of control (Normal and Abnormal) are to be run on a daily basis.
2. If QC results fall outside acceptance criteria, recalibration may be necessary.
3. Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

Linearity:

The Glycosylated hemoglobin procedure shows linearity for GHb levels in the range of 4.0% - 20.0%.

Interferences:

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Method comparison:

Comparative studies were done to compare our reagent with another commercial GHb Assay. No significant differences were observed. Details of the comparative study are available on request.

REFERENCE RANGE

Normal : < 8.0 %
Good control : 8.0 - 9.0 %
Fair control : 9.0 - 10.0 %
Poor control : > 10.0 %

It is recommended that each laboratory establish its own normal range representing its patient population.

NOTES

GHb in whole blood is reported to be stable for one week at 2-8°C. Blood samples with Hemoglobin greater than 18 g/dl should be diluted 1 + 1 with Normal saline before the assay. Samples from patients with Hemoglobinopathies, decreased red cell survival times, gross lipemia may show incorrect results. Do not use Ion Exchange Resin tubes in case of turbidity or visible discolouration. Diabetics with metabolic imbalance may have extremely high levels of the labile aldimine form. In such cases the incubation time during hemolysate preparation may be increased to 15 minutes to ensure elimination of this in stable fraction. Do not use turbid, deteriorated or leaking reagents.

REFERENCES

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3. Bunn,H.F., Diabetes 130, 613 (1981).
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Table for the conversion of Glycosylated Hemoglobin A1 (GHbA1) to Glycosylated Hemoglobin A1c (HbA1c) and to the Mean Blood Glucose level (MBG)

GHbA1	HbA1c	MBG
5.0	3.46	—
5.1	3.54	—
5.2	3.63	—
5.3	3.71	—
5.4	3.79	—
5.5	3.88	—
5.6	3.96	—
5.7	4.04	—
5.8	4.13	—
5.9	4.21	—
6.0	4.30	57
6.1	4.38	60
6.2	4.46	63
6.3	4.55	65
6.4	4.63	68
6.5	4.71	71
6.6	4.80	74
6.7	4.88	77
6.8	4.97	79
6.9	5.05	82
7.0	5.13	85
7.1	5.22	88
7.2	5.30	91
7.3	5.39	93
7.4	5.47	96
7.5	5.55	99
7.6	5.64	102
7.7	5.72	104
7.8	5.80	107
7.9	5.89	110
8.0	5.97	113
8.1	6.06	116
8.2	6.14	118
8.3	6.22	121
8.4	6.31	124
8.5	6.39	127
8.6	6.47	130
8.7	6.56	132
8.8	6.64	135
8.9	6.73	138
9.0	6.81	141
9.1	6.89	144
9.2	6.98	146
9.3	7.06	149
9.4	7.15	152
9.5	7.23	155
9.6	7.31	158
9.7	7.40	160
9.8	7.48	163
9.9	7.56	166
10.0	7.65	169
10.1	7.73	171
10.2	7.82	174
10.3	7.90	177
10.4	7.98	180
10.5	8.07	183
10.6	8.15	185
10.7	8.23	188
10.8	8.32	191
10.9	8.40	194
11.0	8.49	197
11.1	8.57	199
11.2	8.65	202
11.3	8.74	205
11.4	8.82	208
11.5	8.91	211
11.6	8.99	213
11.7	9.07	216
11.8	9.16	219
11.9	9.24	222
12.0	9.32	224
12.1	9.41	227
12.2	9.49	230
12.3	9.58	233
12.4	9.66	236
12.5	9.74	238
12.6	9.83	241
12.7	9.91	244
12.8	9.99	247
12.9	10.08	250
13.0	10.16	252
13.1	10.25	255
13.2	10.33	258
13.3	10.41	261
13.4	10.50	264
13.5	10.58	266
13.6	10.66	269
13.7	10.75	272
13.8	10.83	275
13.9	10.92	278
14.0	11.00	280
14.1	11.08	—
14.2	11.17	—
14.3	11.25	—
14.4	11.34	—
14.5	11.42	—
14.6	11.50	—
14.7	11.59	—
14.8	11.67	—
14.9	11.75	—
15.0	11.84	—
15.1	11.92	—
15.2	12.01	—
15.3	12.09	—
15.4	12.17	—
15.5	12.26	—
15.6	12.34	—
15.7	12.42	—
15.8	12.51	—
15.9	12.59	—
16.0	12.68	—
16.1	12.76	—
16.2	12.84	—
16.3	12.93	—
16.4	13.01	—
16.5	13.09	—
16.6	13.18	—
16.7	13.26	—
16.8	13.35	—