

Quantitative determination of Glycosylated Hemoglobin in Whole Blood
Only for *In Vitro* Diagnostic use

ORDER INFORMATION

REF	Cont.
GHB 10	1 x 10 Tests
GHB 30	1 X 30 Tests

CLINICAL SIGNIFICANCE

Glycosylated Hemoglobin (GHb) is a normal adult hemoglobin (HbA1) which is covalently bonded to a glucose molecule. GHb concentration is dependent on the average blood glucose concentration. It is formed progressively and irreversibly over a period of time and is stable till the life of the RBC. A Single glucose determination gives a value which is true only at the time the blood sample is drawn. GHb on the other hand is unaffected by diet, Insulin or exercise on the day of testing and thus reflects the average glucose level over the last several weeks. Hence, it reflects on the long term metabolic control of glucose in individuals. GHb is now widely recognised as an important test for the diagnosis of Diabetes mellitus and is reliable indicator of the efficacy of therapy. Abraham et al reported excellent correlation between HbA1c (a fraction of HbA1) be used for clinical purpose. There are several acceptable methods of GHb measurement like electrophoresis, ion-exchange chromatography, affinity chromatography, HPLC and colorimetry. GHb kit is based upon the property of non-Glycosylated Hemoglobin to bind with a weak cation exchange resin leaving GHb free in the supernatant.

Method

Photometric test-Ion-Exchange resin Method.

PRINCIPLE

Whole blood is mixed with lysing reagent to prepare a hemolysate. This is then mixed with a weakly binding cation-exchange resin. The non-Glycosylated Hemoglobin binds to the resin leaving GHb free in the supernatant. The GHb percentage is determined by measuring the absorbance of the GHb fraction and of the total Hb.

REAGENT

	10 Tests	30 Tests
01. Resin tubes	10 x 3 ml.	30 x 3 ml.
02. Lysing Reagent	5 ml.	15 ml.
03. Control	0.5 ml.	0.5 ml.
04. Resin Separators	10 no:s	30 no:s

REAGENT PREPARATION

Dissolve the control (3) with 0.5 ml of deionized water by inverting/ swirling. Do not shake vigorously.

REAGENT STORAGE AND STABILITY

Reagent is stable till expiry when stored at 2 - 8°C.
Reconstituted control is stable for 3 days at 2-8°C or 21 days at - 20°C.

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- NaCl solution 9 g/L
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

Whole Blood with EDTA/Heparin

GHb in blood is found to be stable for one week at 2-8°C.

ASSAY PROCEDURE

MANUAL ASSAY PROCEDURE

Assay Temperature	: 23±1°C or 30±1°C
Wave Length	: 415 nm

Step I - Hemolysate preparation

1. Pipette 0.25 ml of lysing reagent (2) in a test tube.
2. Add to it 0.05 ml of well mixed whole Blood/control.
3. Mix well and allow to stand at room temperature for 5 minutes.

Step II - GHb separation and assay

1. Bring Resin Tube (1) to assay temperature by incubating the tube in a water bath.
2. Add to it 0.1 ml of hemolysate (from step1)
3. Position a Resin Separator in the tube, so that the rubber sleeve is approximately 3 cms. above the resin level.
4. Mix the contents on vortex mixer continuously for 5 minutes.
5. Allow the resin to settle at assay temperature for 5 minutes, push down the Resin separator in the tube until the Resin is firmly packed.
6. Pour the supernatant directly into a cuvette and measure the absorbance against deionized water.

Step III - Total Hemoglobin (THb) assay

1. Pipette 5.0 ml of deionized water into a test tube.
2. Add to it 0.02 ml of hemolysate (from step 1).
3. Mix and read absorbance against deionized water.

CALCULATION

Results are calculated, usually automatically by the instrument, as follows:

$$\text{Ghb\%} = \frac{\text{A of GHb}}{\text{A of THb}} \times 7.2 \times \text{temp. factor (Tf)}$$

For assay at 23°C Tf = 1.0; at 30°C Tf = 0.9.

CALBRATORS AND CONTROLS

For the calibration of automated photometric systems the commercially available suitable calibrator is recommended.

The assigned values of this **GHb Standard** have been made traceable to the reference method gas chromatography – isotope dilution mass spectrometry (GC-IDMS).

It is recommended to run a normal and a pathological control serum which is commercially available to verify the performance of the measured procedure. The value of controls should fall within the established limit.

Each laboratory should establish corrective action in case of deviations in control recovery.

PERFORMANCE CHARACTERISTICS

WITHIN RUN

Sample	Mean Concentration	SD	CV %
Level 1	7.55%	0.06	0.08%
Level 2	13.19%	0.06	0.47%

RUN TO RUN

Sample	Mean Concentration	SD	CV %
Level 1	7.63%	0.06	0.75%
Level 2	3.26%	0.54	0.44%

LINEARITY

The method is linear upto a concentration of 20%.

Limit of detection: The limit of detection for Glycosylated Hemoglobin is 2%.

METHOD COMPARISON

A comparison of Accucare Glycosylated Hemoglobin with a commercially available assay (x) using 59 samples gave following results: $R^2 = 0.9900$

REFERENCE VALUES

Non Diabetic	< 8.0 %
Good Control	8.0 - 9.0 %
Fair Control	9.0 - 10.0%

The reference values are to be considered as indicative only. Every laboratory should establish its own normal range.

LIMITATION OF THE PROCEDURE

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

CONVERSION CHART OF GLYCOSYLATED HEMOGLOBIN A1% TO MEAN BLOOD GLUCOSE AND GLYCOSYLATED HEMOGLOBIN A1c%

The glycosylated hemoglobin assay has been validated as a reliable indicator of mean blood glucose (MBG) levels for a period of 8-12 weeks prior to determination. This assay provides valuable information for the physician's clinical assessment of long term diabetic control. Physicians have conventionally used information such as symptoms, urine tests and random blood glucose determination to evaluate the metabolic state of their diabetic patient and to estimate roughly the average blood glucose of the patient. Recently, the glycohemoglobin test has been shown to have a linear correlation with MBG results from patients performing frequent self monitoring of blood glucose levels. Using this correlation, a table of the glycosylated Hemoglobin A1% from the Glycosylated Hemoglobin assay A1c% & Mean Blood Glucose is obtained.

A1	A1c	MBG	A1	A1c	MBG
6.0	4.30	35	9.9	7.56	178
6.1	4.38	39	10.0	7.64	182
6.2	4.46	43	10.1	7.73	186
6.3	4.54	46	10.2	7.81	189
6.4	4.63	50	10.3	7.89	193
6.5	4.71	54	10.4	7.98	197
6.6	4.79	58	10.5	8.06	200
6.7	4.88	61	10.6	8.15	204
6.8	4.96	65	10.7	8.23	207
6.9	5.05	68	10.8	8.31	211
7.0	5.13	72	10.9	8.40	215
7.1	5.21	76	11.0	8.48	219
7.2	5.30	79	11.1	8.56	222
7.3	5.38	83	11.2	8.65	226
7.4	5.46	87	11.3	8.73	230
7.5	5.55	90	11.4	8.82	233
7.6	5.63	94	11.5	8.90	237
7.7	5.72	98	11.6	8.98	241
7.8	5.80	101	11.7	9.07	244
7.9	5.88	105	11.8	9.15	248
8.0	5.97	109	11.9	9.24	252
8.1	6.05	112	12.0	9.32	255
8.2	6.14	116	12.1	9.40	259











8.3	6.22	120	12.2	9.49	263
8.4	6.30	123	12.3	9.57	266
8.5	6.39	127	12.4	9.65	270
8.6	6.47	131	12.5	9.74	274
8.7	6.55	134	12.6	9.82	277
8.8	6.64	138	12.7	9.91	281
8.9	6.72	142	12.8	9.99	285
9.0	6.81	145	12.9	10.07	288
9.1	6.89	149	13.0	10.16	292
9.2	6.97	153	13.1	10.24	295
9.3	7.06	156	13.2	10.33	299
9.4	7.14	160	13.3	10.41	304
9.5	7.22	164	13.4	10.49	309
9.6	7.31	167	13.5	10.58	314
9.7	7.39	171	13.6	10.66	320
9.8	7.48	175	13.7	10.74	326

It should be noted that glycosylated hemoglobin values below 6.0% may indicate the presence of abnormal hemoglobins (S and C) or hemolytic disorders. To rule out the presence of abnormal hemoglobins or hemolytic disorders additional tests are recommended.

BIBLIOGRAPHY

- Trivelli, LIA. et al (1971) New Eng. J. Med. 284:353.

GLOSSARY OF SYMBOL

	Consult Instruction for Use		Lot Number
	Catalog Number		Date of Manufacturing
	Store between		Use By or Expiration Date
	Manufacturer		For <i>in vitro</i> Diagnostic use only
	Keep away from sunlight		Content of the kit



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