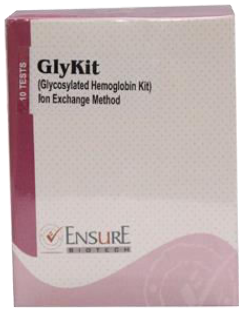
GLYKIT

(GLYCO-HEMOGLOBIN KIT)



PRINCIPLE

A hemolyzed preparation of the whole blood is mixed for Five minutes with cation-exchange resin. HbAo binds to the resin. After the mixing period, a litter is used to separate the supernatant containing the glycohemoglobin from the resin. (Note: This binding is temperature dependent.) The percent glycohemoglobin is determined by measuring the absorbance at 415nm (405-420nm) of the glycohemoglobin fraction and the total hemoglobin fraction. The ratio of the two absorbances gives the percent glycohemoglobin.

CLINICAL SIGNIFICANCE

Throughout the circulatory life of the red cell, glycohemoglobin is formed continuously by the adduction of glucose to the N-terminal of the hemoglobin beta chain. This process, which is non-enzymatic, reflects the average exposure of hemoglobin to glucose over an extended period. The non-glycosylated hemoglobin, which consists of the bulk of the hemoglobin has been designated HbAo. The present glycohemoglobin procedure employs a weak binding cation exchange resin for the rapid separation of glycohemoglobin (fast fraction) from nonglycosylated hemoglobin. Over 80% of the labile fraction of glycohemoglobin is removed during the separation step in this procedure.

SAMPLE COLLECTION & STORAGE:

- Venous blood with EDTA using aseptic technique
- Glycohemoglobln in whole blood is stable for one week at 2-8°C.

PRECAUTIONS

* **GLYKIT** is for in vitro diagnostic use only.
* Lysing reagent contains cyanide (poison). Do not ingest.
* it has been reported that bilirubinemia may interfere with ion. exchange methods
* Blood samples with total hemoglobin greater than 18 g/dl should be diluted x 2 with deionized water before assay

KIT CONTENTS & STORAGE

	1OT
Pre filled resin tubes	10nos
Serumi/Resin separators	10nos
Lysing Reagent	1Vial

All reagents are to be stored at 2-8°C and stable till expiry date stated on the labels.

SYSTEM PARAMETERS

Reaction Type	End point
Wave length	405nm
Flow Cell Temp	30°C
Units	%
Incubation	5+5 minutes
Zero Setting	Dist Water

PROCEDURE

A. HEMOLYSATE PREPARATION

Pipette in clean dry test tubes labeled as Control(C) and Test(T)

	Test
Lysing Reagent	0.25 ml
Sample control	50µl

Mix well and keep at RT for 5 minutes.Now this Hemolysate is ready for use in step B

B. GLYCOHEMOGLOBIN (GHB) PREPARATION

1. Before use, mix the resin tube by inverting 10 times and bring it to assay temperature by placing in a water bath adjusted to 23°C or 30°C
2. Add 100ul of the hemolysate (from step A) to resin tube.
3. Position the filter separators in the tubes so that the rubber sleeve is 1cm above the liquid level.

4. Place the tubes on the rotator and mix continuously for 5 minutes.
5. Remove the tube from the rotator & push the filter separators into the tubes until the resin is tightly packed.
6. The supernatant may be poured into another tube or directly into a cuvette for measurement.
7. Zero the instrument at 405 nm (405-420nm) with dist. water & read the results

C. TOTAL HEMOGLOBIN (THE)PREPARATION

1. Dispense 5.0 ml deionized water into tubes labeled: Test Control.

2. Place 20ul of the hernolysate (from step A) into the test tubes and Mix.
3. Adjust the instrument to zero absorbance at 415nm (405-420nm) with deionized water as the blank and read results

NOTE: This assay should be performed at 23°C or 30°C. Samples should be read within an hour before evaporation becomes significant.

CALCULATIONS

GHB is sample (%):
(Abs of Glyo hemoglobin (GHb) / Abs of Total hemoglobin (THb)) x 4.6 x Temp. Factor (Tf

For an assay at 23^0cTf=1.0
For an assay at 30^0cTf=0.9

LINEARITY

The assay shows linearity for GHb levels in the range of 4.0% - 20%

NORMAL RANGE

Non diabetic	-	5.0-8.0%
Good control	-	8.0-9.0%
Fair control	-	9.0-10.0%
Poor control	-	10.0 %& above

Each laboratory should establish its own expected values. In using glycated Hb to monitor diabetic patients, results should be interpreted individually. That is, the patient should be monitored against him or herself. There is a 3-4 week tune lag before % glycohemoglobin reflects changes in blood glucose level

BIBILOGRAPHY

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