Glycosylated Hemoglobin(GHb)Assay Kit

(Cat/No.:BC109 Size:15T/14S)

1.Assay principle:

Glycosylated hemoglobin (GHb) contains ketoamine bond. If GHb is heated in acidic solution, then its hexose part will be dehydrated to produce 5-hydroxymethyl furfural (5-HMF). 5-HMF reacts with TBA to appear yellow color, then it is able to calculate GHb content by measuring OD values.

2. Composition (The kit is valid for 3 months)

Reagent 1: Liquid 20ml×1 bottle, can be stored at room temperature for 6 months.
Reagent 2: Protein precipitant 20ml×1 bottle, can be stored at room temperature for 6 months.
Reagent 3: Chromogenic agent 10ml×1 bottle, can be stored at room temperature away from light for 3 months.
Reagent 4: Cyanmethemoglobin determination solution 40ml ×1 bottle, can be stored away from light for 3 months.

3. Operation procedure

(1) RBC washing: Transfer 2~4ml anticoagulated (by EDTA or heparin) whole blood in scaled centrifuge tube, centrifugate at 500~1000 rpm for 5~10 minutes, remove supernatant and keep RBC sediment, wash RBC by physiological saline according to the method above 2~3 times.
* If you can not wash immediately, then this blood can be stored for 48 hours.

(2) Hemolysate preparation:

① Take 1ml packed red cells, add 1.5ml cold distilled water, shake test tube by your hand rapidly for several minutes or mix sufficiently by vortex for 1 minute in order to prepare hemolysate. This hemolysate can be stored at -20℃ for 70 days.

② Hemolysate Hb assay:

Take 10μl hemolysate, add 2.5ml Reagent 4, mix sufficiently, place at room temperature for 10 minutes, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 540nm.
Measured OD value×0.3677= Hb content (g/ml)

(3) Acidification

Take glass test tubes, label them ("O" for blank tube, "U" for sample tubes), add 2ml in "O" tube, add 2ml hemolysate in "U" tubes, then add 1ml Reagent 1 in each tube for acidificantion. Please keep adding slowly, shake during adding.

(4) Hydrolysis

Seal test tubes above by rubber stopper or plastic membrane (pierce a small hole by needle and then tight by rubber band). Heat in boiling water bath or 100℃ loft drier to hydrolyze for 1 hour.

(5) Chromogenic reaction

① Add 1ml Reagent 2 (protein precipitant) in each tube (hemolysate may freeze in cold days, you can heat it to thaw before use). Please add Reagent 2 slowly and keep shaking during adding. Mix sufficiently by vortex, centrifugate at 3000~3500rpm for 10 minutes.

②Take 2ml supernatant from each tube, add 0.5ml Reagent 3 (chromogenic agent) separately, place in 40℃ water bath for 30 minutes.
③ Cool to room temperature, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 443nm (ajust zero by distilled water).

4. Calculation

(1) Formula:GHb's result is expressed by "OD value per 10g Hb".

$$\text{OD value per 10g Hb} = \frac{OD_{Sample} - OD_{Blank}}{\text{Hb content (g) in 2ml hemolysate}} \times \text{Dilution times} \times 10g$$

$$= \frac{OD_{Sample} - OD_{Blank}}{\text{2ml hemolysate} \times \text{Hbg/ml}} \times 2 \times 10$$

$$= \frac{OD_{Sample} - OD_{Blank}}{\text{Hb content (g)/ml hemolysate}} \times 10$$

(2) Example:

Take 1ml packed red cells, add 1.5ml distilled water to make hemolysate, in results, Hb content is 0.108g/ml, Ghb sample tube's OD value is 0.336, Blank tube's OD value is 0.006, calculate as follows:

$$\frac{0.336-0.006}{2 \times 0.108} \times 2 \times 10 = 30.6$$

5. Referenced value

At normal condition, GHb per 10g Hb 's OD value is 13.3~23.5.

6. Appendix

(1) This method has good accurancy, its average CV is 4.2%.

(2) If you can not do GHb assay immediately, then this hemolysate can be stored for 70 days, it will not affect result.

(3) This method is convenient, doesn't require special equipment.