Glycosylated Hemoglobin (GHb/HbA1c) Colorimetric Assay Kit

Catalog No: E-BC-K089-M
Method: Colorimetric method
Specification: 15 Assays

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA)   Fax: 240-252-7376(USA)
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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.
Application
This kit can be used to measure Glycosylated Hemoglobin (GHB/HbA1c) content in animal red blood cells, whole blood and other samples. This kit (15 Assays) can detect 14 samples.

Detection significance
Glycosylated hemoglobin Alc (HbA1c) is an index that reflects control of blood glucose in diabetes for a long time (4-10 weeks). Poor long-term control of blood glucose will cause increased content of glycosylated hemoglobin. So determination of HbA1c can help to control the blood glucose in diabetics and it plays an important role in the study of peripheral vascular and cardiovascular complications of diabetes.

Detecting principle
The glycosylated hemoglobin with ketoamine bond in hemoglobin is heated in acidic environment, and hexose dewatered partially to form 5-hydroxymethylfurfural (5-HMF). 5-HMF can react with TBA to form a yellow complex which can be detected by colorimetric assay at 443 nm.

Kit components

<table>
<thead>
<tr>
<th>Component</th>
<th>Specification</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1 Liquid</td>
<td>20 mL × 1 vial</td>
<td>RT, 6 months</td>
</tr>
<tr>
<td>Reagent 2 Protein precipitant</td>
<td>20 mL × 1 vial</td>
<td>RT, 6 months</td>
</tr>
<tr>
<td>Reagent 3 Chromogenic agent</td>
<td>10 mL × 1 vial</td>
<td>RT, 3 months, shading light</td>
</tr>
<tr>
<td>Reagent 4 Ferric cyanide hemoglobin detection buffer</td>
<td>40 mL × 1 vial</td>
<td>RT, 3 months, shading light</td>
</tr>
</tbody>
</table>

Experimental instruments
Test tube, Micropipettor, Vortex mixer, 100℃ water bath, Centrifuge, Spectrophotometer (443 nm)

Operation Steps
1. The washing of RBC:
   Take EDTA or heparin anticoagulant whole blood 2-4 mL and place in a graduated centrifuge tube, centrifuge for 5-10 min at 500-1000 rpm. Discard the supernatant and keep the erythrocyte sedimentation, then wash with normal saline 2-3 times as described above. (Or stand for 48 hours if there is no time to wash anticoagulant whole blood).

2. Preparation of hemolysis:
   (1) Take 1 mL hematocrit red blood cells and add 1.5 mL cooling double distilled water, shake intensely with hands for a few minutes, or mix thoroughly by vortex mixer for one minute to get hemolysis, the hemolysis can be stored at -20℃ for 70 days.
   (2) Hemoglobin (Hb) concentrations of hemolysis are measured as follows:
   Take 10 μL hemolysis and add 2.5 mL Reagent 4, mix fully and stand for 10 min at room
temperature. Set to zero with double-distilled water and measure the OD value of each tube at 540 nm with 1 cm optical path cuvette. Multiply the above absorbance values by 0.3677 to get the Hb content (g/mL).

3. **Acidification:**
Take glass test tubes and mark "0" as blank tube and "U" as measured tube. Add 2 mL double-distilled water into the blank tube, while add 2 mL hemolysis into measured tube, then add 1 mL Reagent 1 into each tube for acidification. Reagent 1 should be added slowly, and shake tubes during the dropping process.

4. **Hydrolysis:**
Attach the above test tubes with rubber stoppers or plastic film, (poke a small hole with a needle and then tie with the rubber band, seal the glass tube mouth). Hydrolysis for one hour at boiling water bath or 100°C oven.

5. **Color development:**
   (1) Add 1 mL Reagent 2 (protein precipitant) to each tube (In case of cold weather, the hydrolyzate may be solidified, and it should be dissolved by heating). Reagent 2 should be added slowly, and shake tubes during the dropping process. Then mix by vortex shaker, centrifuge for 10 min at 3000-3500 rpm.
   (2) Take 2 mL supernatant, add 0.5 mL Reagent 3 (chromogenic agent) each tube, incubate at 40°C water bath for 30 minutes.
   (3) After cooling, set to zero with double-distilled water and measure the OD value of each tube at 443 nm with 1 cm optical path cuvette.

**Calculation of results**

※ The content of GHb is expressed in terms of absorbance per 10 g hemoglobin.

\[
\text{GHb content} = \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}}{\text{Hemoglobin content in 2mL hemolysis}} \times \text{Dilution factor} \times 10 \text{ g}
\]

\[
= \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}}{\text{Hemoglobin content / the volume of hemolysis}} \times 10
\]

**Notes**

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of the kit is 3 months.
4. Do not use components from different batches of kit.