Glycated Hemoglobin (GHb) Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC5615

Size:100T/96S

Components:

Reagent I: Liquid 25mL×1. Store at 2-8℃.

Reagent II: Liquid 14mL×1. Store at 2-8℃.

Reagent III: Liquid 14mL×1. Store at 2-8℃.

Reagent IV: Liquid 6mL×1. Store at 2-8℃.

Standard: Powder×1. Store at 2-8℃. Add 1mL distilled water to form 10mg/mL standard solution. It could be stored at 2-8℃ for four weeks. Before use, mix 50μL 10mg/mL standard solution and 750μL distilled water to prepare a standard solution of 0.625 mg/mL.

Product Description:

Glycated hemoglobin (GHb) is formed when a non-enzyme reaction occurs between glucose in the serum and hemoglobin in the erythrocyte. The amount of GHb generated is proportional to the mean blood glucose during the 8–10 weeks before the test. GHb is composed of HbA1a, HbA1b and HbA1c. HbA1c accounts for about 70% and its structure is relatively stable. GHb content is used as a biomarker for diabetes mellitus control.

The hexose moiety of GHb is converted to 5-hydroxymethylfurfural (5-HMF) by heating at 100℃ in the presence of a weak acid. 5-HMF reacts with Thiobarbituric Acid (TBA) to produce colored compound, which has an absorption peak at 443 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, desk centrifuge, water-bath, balance, transferpettor, micro glass cuvette/96 well plate, ice and distilled water.

Procedure:

I. Sample preparation

1. Red blood cell preparation: take anticoagulated whole blood, centrifuge it at 1000-3000rpm, 4℃ for 10min, leave the layer of red blood cells, add saline, gently invert it and mix it, centrifuge it again, leave the layer of red blood cells, and then add saline to repeat the washing for 1-2 times, and then get the red blood cells for the preparation of lysed blood.
2. Preparation of lysed blood: take red blood cells, add appropriate amount of cold distilled water, vortex well and mix well to get lysed blood.

Note: At least 950 μL is required for one assay.

II. Determination

A. Total Hb content

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1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 400 nm and set spectrophotometer counter to zero with distilled water.
2. Add reagents in 1.5mL EP tube as the following:

Reagent (μL)	Blank tube1 (A_{B1})	Test tube (A_{To})	Standard tube (A_S)
Distilled water	50	-	-
Sample	-	50	-
Standard	-	-	50
Reagent I	200	200	200
Mix thoroughly and stand at room temperature for 5min. Add 200μL mixture into micro glass cuvette/96 well flat-bottom plate and detect the absorbance value at 400 nm, recording as A_{B1}, A_{To}, and A_S. $\Delta A_{To}=A_{To}-A_{B1}$. $\Delta A_S=A_S-A_{B1}$. Blank tube1 and standard tube need to test once or twice.			

B. GHb content

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 443 nm and set spectrophotometer counter to zero with distilled water.
2. Add reagents in 1.5mL EP tube as the following.

Reagent (μL)	Blank tube2 (A_{B2})	Test tube (A_T)
Distilled water	250	-
Sample	-	250
Reagent II	125	125
Add reagent II slowly, mix well, bath at 100℃(wrapped with sealing film to prevent bursting of the lid)for one hour and cool to room temperature		
Reagent III	125	125
Add reagent III slowly, mix well, centrifuge at 3500g for 10min at room temperature and take supernatant		
Supernatant	200	200
Reagent IV	50	50
Mix thoroughly and bath at 40℃ for 30min. Cool to room temperature. Add mixture into micro glass cuvette/96 well flat-bottom plate and detect the absorbance value at 443 nm, recording as A_{B2}, A_T. $\Delta A_T=A_T-A_{B2}$. Blank tube2 needs to test once or twice.		

III. GHb content calculation:

1. Total Hb content calculation:

Total Hb content (g/mL) = $\Delta A_{To}\div(\Delta A_S\div C_S)\times F = 6.25\times10^{-4}\times\Delta A_{To}\div\Delta A_S\times F$

C_S: Standard concentration, 0.625 mg/mL=6.25×10^{-4}g/mL;

F: Dilution factor.

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2. GHb content calculation:

GHb content was reflected as the absorbance per 10 g of hemoglobin:

The absorbance per 10 g of Hb=$\Delta A_T \div (C_{Hb} \div 2 \times V_S) \times 10 \times F = 25 \times \Delta A_T \div C_{Hb} \times F$

C_{Hb}: Total Hb content of sample, g/mL;

Vs: Added supernatant volume in the process of GHb content measurement, 0.2 mL;

2: Dilution factor;

F: Dilution factor;

10: Hemoglobin weight, 10 g.

3. Calculation results conversion formula:

A. micro glass cuvette:

IFCC-HbA1c (%) = (The absorbance per 10 g of Hb×0.001+0.0154) ×100%

IFCC-HbA1c (mmol/mol) =1300×IFCC-HbA1c (%) ×100 -7.4 (This formula should be calculated by converting to a decimal divided by 100)

DCCT-HbA1c (%) =((IFCC-HbA1c(mmol/mol) ÷10.929+2.15) ÷100) ×100%

B. 96-well flat-bottom plate:

IFCC-HbA1c (%) =(The absorbance per 10 g of Hb×0.0005+0.0154)×100%

IFCC-HbA1c (mmol/mol) =13×IFCC-HbA1c (%) ×100 -7.4

DCCT-HbA1c (%) =((IFCC-HbA1c(mmol/mol) ÷10.929+2.15) ÷100) ×100%

Note:

1. It is recommended to use spiral EP tubes with lids in the boiling water bath for GHb content determination. If ordinary EP tubes are used, they should be wrapped with multiple layers of sealing film to avoid the EP tube lids from collapsing during the boiling water bath.
2. When adding reagent III should be mixed while adding reagent, after adding part of reagent III may appear the phenomenon of reaction solution solidification, need to be stirred before centrifugation.
3. If ΔA_{To} is more than 1.0, it is recommended to dilute the sample with distilled water before determination. If ΔA_{To} is less than 0.01 or close to A_B, it is recommended to increase added sample volume before determination. And modify the added volume of blank tube and standard tube at the same time. And modify the calculation formula.
4. If ΔA_T is more than 1.0, it is recommended to dilute the sample with distilled water before determination. If ΔA_T is less than 0.01 or close to A_B, it is recommended to increase added sample volume before determination. And modify the added volume of blank tube and standard tube at the same time. And modify the calculation formula.

Experimental example:

1. (1) Take 50μL rabbit hemolytic blood and dilute it 50 times with distilled water. Then operate according to the determination steps, calculate $\Delta A_{To}=A_{To}-A_{B1}$=0.992-0.053=0.939,

$\Delta A_S = A_S - A_{B1}$ =0.348-0.053=0.295. The result is calculated:

Total Hb contant (g/mL) = $6.25\times10^{-4}\times\Delta A_{To}\div\Delta A_S\times F$ =0.0995 g/mL.

(2) Take 250μL rabbit hemolytic blood and operate according to the determination steps, calculate $\Delta A_T = A_T - A_{B2}$=0.160-0.048=0.112. The result is calculated:

The absorbance per 10 g of Hb==$25\times\Delta A_T\div C_{Hb}$=112.563.

References:

[1] Parker K M, England J D, Da Costa J, et al. Improved colorimetric assay for glycosylated hemoglobin [J]. Clinical Chemistry, 1981, 27(5): 669-672.

[2] Murray B A, Walsh D J, Fitzgerald R J. The Advances of Detection Technology and Clinical Application of Glycated Hemoglobin [J]. Medical Recapitulate, 2013, 19(14): 2602-2605.

Related Products:

BC1730/BC1735	Serum Ferri Ion Content Assay Kit
BC5580/BC5585	Hemoglobin (Hb) Content Assay Kit
BC5590/BC5595	Free Hemoglobin (FHb) Content Assay Kit
BC5600/BC5605	Methemoglobin (MetHb) Content Assay Kit

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