CheKine™ Micro Glycosylated Hemoglobin (GHb) Content Assay Kit

Cat #: KTB2225 Size: 48 T/48 S 96 T/96 S

	Micro Glycosylated Hemoglobin (GHb) Content Assay Kit		
REF	**Cat #:** KTB2225	LOT	**Lot #:** Refer to product label
	Applicable samples: Anticoagulated whole blood		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Glycosylated hemoglobin (GHb) is a form of hemoglobin (Hb) present in human blood, resulting from the slow, sustained, and irreversible non-enzymatic protein glycation reaction between the amino-terminal end of the valine residue on the β-chain of hemoglobin in red blood cells and glucose. Given that the lifespan of red blood cells in the human body is approximately 120 days, the level of GHb reflects the average blood glucose levels over the preceding 2–3 months; consequently, it has now been widely regarded as an effective biomarker for assessing glycemic control in patients with diabetes mellitus.

CheKine™ Micro Glycosylated Hemoglobin (GHb) Content Assay Kit can detect samples such as anticoagulated whole blood. The detection principle of this assay kit is that the sugars on glycated hemoglobin, when heated under acidic conditions, undergo dehydration condensation to form 5-hydroxymethylfurfural (5-HMF); subsequently, 5-HMF reacts with TBA (Thiobarbituric Acid) to produce a colored compound exhibiting a characteristic absorption peak at 443 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	10 mL	20 mL	4℃
Reagent Ⅱ	15 mL	30 mL	4℃, protected from light
Reagent Ⅲ	7.5 mL	15 mL	4℃
Reagent Ⅳ	3 mL	6 mL	4℃, protected from light
Standard	1 mL	1 mL	4℃, protected from light

Materials Required but Not Supplied

·Microplate reader or visible spectrophotometer capable of measuring absorbance at 400 nm and 443 nm
·96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 2 mL screw-cap EP tube
·Water bath, constant temperature incubator, ice maker, centrifuge
·Deionized water, normal saline

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Reagent Preparation

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent Ⅲ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅳ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Standard: Ready to use as supplied. Equilibrate to room temperature before use. 10 mg/mL hemoglobin standard. Store at 4℃, protected from light.

0.625 mg/mL Standard: Prepare 0.625 mg/mL of hemoglobin standard by diluting 10 μL 10 mg/mL hemoglobin standard into 150 μL deionized water. Thoroughly mix and use immediately after preparation.

Sample Preparation

Note: We recommend that you use fresh anticoagulated whole blood. If immediate processing is not possible, the blood may be stored at 4 ℃ for up to 48 hours. When rinsing with normal saline, gently invert and mix the sample to avoid hemolysis. When measuring total hemoglobin, it is recommended to dilute the blood sample 100–300–times.

Anticoagulated whole blood: Use fresh anticoagulated whole blood, centrifuge at 4 ℃ and 3,000 rpm for 10 min; discard the supernatant and retain the red blood cell (RBC) pellet. Add 1 mL of normal saline, gently invert and mix thoroughly, then centrifuge again at 4 ℃ and 3,000 rpm for 10 min; discard the supernatant and retain the RBC pellet. Repeat the saline washing step twice; the resulting precipitate constitutes the RBCs, which will be used to prepare the hemolysis sample. Combine the RBCs with pre-cooled deionized water in a 1:2 ratio, and vortex vigorously to homogenize, thereby obtaining the hemolysis mixture.

Assay Procedure (Total Hemoglobin)

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 400 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent (μL)	Test Well (μL)	Blank Well 1 (μL)	Standard Well (μL)
Sample	40	0	0
Deionized Water	0	40	0
0.625 mg/mL Standard	0	0	40
Reagent Ⅰ	160	160	160

3. After thorough mixing, incubate for 5 min at 25℃. The absorbance value is measured at 400 nm. The test well is marked as A_{Total}, the blank well 1 is recorded as $A_{Blank\ 1}$, the standard well is marked as $A_{Standard}$. Finally calculate $\Delta A_{Total}=A_{Total}-A_{Blank\ 1}$. $\Delta A_{Standard}=A_{Standard}-A_{Blank\ 1}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Total} is less than 0.01 or approaches the absorbance value of the blank tube, it is recommended to increase the sample volume and re-measure. If ΔA_{Total} is greater than 1.0, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Assay Procedure (Glycated Hemoglobin)

Note: When adding Reagent Ⅱ and Reagent Ⅲ, ensure they are added slowly; during the boiling water bath treatment, keep the container sealed to prevent moisture loss. After adding Reagent Ⅲ, the reaction mixture may solidify; therefore, it should be rapidly vortexed to ensure thorough mixing.

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 443 nm.

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Visible spectrophotometer was returned to zero with deionized water.

2. The blank tube requires only a single measurement; perform the following procedure in a 2 mL screw-cap EP tube.

Reagent (μL)	Test tube (μL)	Blank tube 2 (μL)
Sample	250	0
Deionized Water	0	250
Reagent Ⅱ	125	125
Add Reagent Ⅱ slowly, mix thoroughly, place in a boiling water bath for 1 h, and cool to room temperature.		
Reagent Ⅲ	125	125
Add Reagent Ⅲ slowly, then immediately mix thoroughly; centrifuge at 3,500 g for 10 min at room temperature (25℃), and transfer the supernatant to a 1.5 mL EP tube.		
Supernatant	200	200
Reagent Ⅳ	50	50

3.After thorough mixing, incubate at 40℃ for 30 min; once cooled to room temperature, transfer 200 μL of the reaction mixture into a 96-well plate, measure the absorbance at 443 nm. The test Well is marked as $A_{Test,}$ the Blank Well 2 is recorded as $A_{Blank\ 2}$, Finally calculate $\Delta A_{Test}=A_{Test}-A_{Blank\ 2}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01 or approaches the absorbance value of the blank tube, it is recommended to increase the sample volume and re-measure. If ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted with Deionized Wate, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculation of GHb content in the sample

Calculation of total hemoglobin (Hb) content

Hb (g/mL)=$\Delta A_{Total}\div(\Delta A_{Standard}\div C_{Standard})\times n$**=$6.25\times10^{-4}\times\Delta A_{Total}\div\Delta A_{Standard}\times n$**

Calculation of total Glycated Hemoglobin (GHb) content. (The glycated hemoglobin content is expressed as the absorbance value per 10 g of hemoglobin.)

Absorbance per 10 g of hemoglobin=$\Delta A_{Test}\div(C_{Hb}\div2\times V_{Sample})\times10\times n$**=$100\times\Delta A_{Test}\div C_{Hb}\times n$**

$C_{Standard}$: concentration of the standard, 0.625 mg/mL = 6.25×10^{-4} g/mL; C_{Hb}: total hemoglobin content in the sample, g/mL; 2: GHb quantification step – the sample is diluted 2-times during the transfer to supernatant; V_{Sample}: volume of super natant added in the GHb quantification step, 0.2 mL; 10: hemoglobin mass, 10 g; n: dilution factor.

2. onversion formula for calculation results

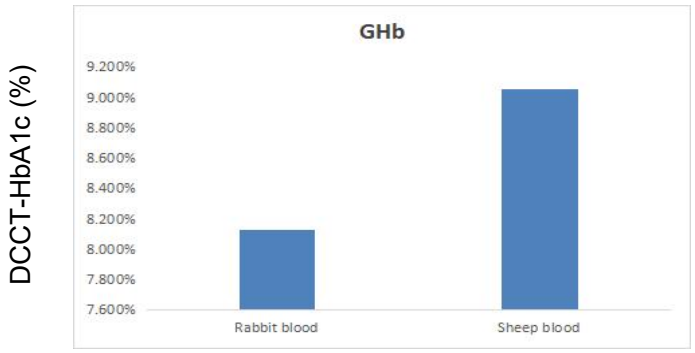
IFCC-HbA1c (%)=（Absorbance per 10 g of hemoglobin×0.0005+0.0154）×100%

IFCC-HbA1c (mmol/mol)=1300×IFCC-HbA1c (%)÷100-7.4

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

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Typical Data



Recommended Products

Catalog No.	Product Name
KTB2226	CheKine™ Micro Glycosylated Serum Protein (GSP) Content Assay Kit
KTB1330	CheKine™ Micro Blood Glucose Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.

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