Glycohemoglobin A1c (HbA1c) Test Kit

Enzymatic Method

[Production]
Glycohemoglobin A1c (HbA1c) Test Kit

[Application]
In vitro test for the quantitative determination of HbA1c concentration in human whole blood on photometric systems. HbA1c is a product of hemoglobin (Hb) that produces a slow and continuous non-enzymatic glycation reaction under the action of high blood glucose. Glucose modifies hemoglobin specifically in its n-terminal valine residue to form glycated hemoglobin. Under normal physiological conditions, the production of non-enzymatic glycosylation reaction products is positively proportional to the concentration of reactants. Since hemoglobin concentration remains relatively stable, glycosylation levels are mainly dependent on glucose concentration and are also related to the length of hemoglobin and glucose exposure. Therefore, HbA1c is a good indicator for the average blood glucose level of patients at 2 to 3 months.

[Principle]
In response to proteases, the n-terminus of the hemoglobin A1c β chain is severed and glycosylated dipeptides are released. The Hb concentration can be obtained by measuring the absorbance of 505nm in the first reaction. In the second reaction, fructose-based peptide oxidase (FPOX) acts on glycosylated dipeptide to produce hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with chromogenic agent, and the concentration of HbA1c can be calculated by measuring the absorbance at 660nm. According to the HbA1c concentration and Hb concentration calculated HbA1c %.

[Reagents]

Components	**Concentrations**
Reagentl (R1):	
Tris Buffer	100mmol/L
PRK	500ku/L
DA-67	10mmol/L
Reagents 2 (R2) :	
FPOX	50KU/L
Good's Buffer	100mmol/L
Sample Treatment Solution	
Good's Buffer	100mmol/L

[Sample Requirements]
1. No hemolytic anticoagulation whole blood, use EDTA anticoagulation.
2. In the sample, bilirubin≤40mg/dL, carbamyl Hb≤7.5mmol/L, acetylationHb≤5.0mmol/L, chylomicron ≤200 turbidity unit, VC≤50mg/dL.

[sample treatment]

Use whole blood directly(do not centrifuge the sample),Take 25μL of whole blood and add it into 500μL sample solution. After blending, the samples were measured on the automatic biochemical analyzer.

[Calibrator Preparation]
Carefully open the bottle, avoiding the loss of lyophilizate, and pipette in exactly 1.0 mL of sample solution. Carefully close the bottle and dissolve the contents completely by occasional gentle swirling within 30 minutes. Avoid the formation of foam. The dissolved calibrator can be used without any other pretreatment.

[Quality control Preparation]
Carefully open the bottle, avoiding the loss of lyophilizate, and pipette in exactly 1.0 mL of sample solution. Carefully close the bottle and dissolve the contents completely by occasional gentle swirling within 30 minutes. Avoid the formation of foam. The dissolved control can be used without any other Pretreatment.

[Method]
1. Reagent preparation: Liquid reagent can be used when opened
2. Measurement:
(1)Two items were not measured simultaneously

Main wavelength	500nm	Subwavelength /
Temperature	37 °C	Type 2-Endpoint
Sample (calibration)		12μL
R1		180μL
Mix and keep at 37°C, 5 min, determination of absorbance A.		

① Detection of hemoglobin
ΔA=A sample / calibrator -A blank

②Detection of glycosylated hemoglobin

Main wavelength	660nm	Sub wavelength 800nm
Temperature	37°C	Type 2-Endpoint
Sample (calibration)		12μL
R1		180μL
Mix and keep at 37°C, 5 min, determination of absorbance A1.		
R2		60μL
Mix and keep at 37°C, 5 min, determination of absorbance A2.		

ΔA=[(A2-A1) sample/calibrator]-[(A2-A1) blank]

(2)Two items were measured simultaneously

Method:

Sample		reagent R1	37°C	measure
12 μ L	+	180 μ L	→ 5min	ABS I
		reagent R2	37°C	measure
		60 μ L	→ 5min	ABS II
			→calculated concentration	

ABS I : Difference of absorbance between 505nm and 800nm

ABS II : Difference of absorbance between 660nm and 800nm

Iy: The apparatus must meet the following conditions and two items were measured simultaneously

①Different test items can correspond to the same reagent site.

② The absorbance at different wavelengths of the same reaction cup can be measured simultaneously.

[Calculation]

The results of HbAlc depends on the concentration of hemoglobin and glycated hemoglobin were expressed directly by HbAlc%. In order to cope with the standardized value of HbAlc recommended by different agencies, the inter-project calculation formula of automatic analyzer should be used:

Method of National glycosylated hemoglobin standardization program / method of diabetes control and complications study (NGSP/DCCT):

HbAlc=91.5*HbAlc (pmol/L) /Hb (pmol/L) +2.15

[Calibration]

l.It is recommended to use the calibrator in kits for tow-point calibration. 2.The calibrator values assigned by BioSino standard transfer procedure and routine method are listed in the value sheet. The traceability process is based on ISO 175112, This method has been standardized against the approved IFCC reference method for the measurement of HbAlc in human blood and can be transferred to results traceable to DCCT/NGSP by calculation.

3.Calibration frequency:

After reagent lot changed.

As required following quality control procedures.

[Quality control]

At least two levels of control material should be analyzed with each batch of samples. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. We recommend using the Control in kits to verify the performance of the measurement procedure; other suitable control material can be used in addition. Each laboratory should establish its own internal qualify control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

[Storage and stability]

Up to expiration date indicated on the label, when stored unopened at 2-8 °C and protected from light.

Once opened, the reagents are stable for 28 days when refrigerated on the analyzer or refrigerator.

Contamination of the reagents must be avoided. Do not freeze the reagents.

Once dissolved, the calibrator are stable for 7 days at 2 - 8 ˚C, the control are stable for 7 days at 2 - 8 °C , do not freeze.

[References]

1. Under the conditions of 37 °C , 660nm wavelength and lcm light diameter, the change of blank absorbance of reagent should not exceed 0.10 when purified water is used as sample for reagent test.
2. Precision: repeatability CV is less than 5%; The relative range R between batches shall not exceed 10%.
3. Accuracy: test traceable standard products; Within 3%-16%, the relative deviation should not exceed ±10%.
4. Refrence Range: **Normal Glucose Tolerance ≤5.6%**
   Pre-diabetics (High risk for diabetes) 5.7-6.4%
   Diabetes ≥6.5%
5. Linear range: 1) When Hb is in the range of 90-300 u mol/L, the percentage of glycosylation is in the range of 3%-16%, and the correlation coefficient r should not be less than 0.990; The relative deviation between the measured concentration and the estimated value should not exceed ±10%.
6. Analytical sensitivity: when testing HBA1C with this reagent, the absorbance change caused by 10 u mol/L concentration is not less than 0.05.
7. Stability: All dosage forms of reagents can be used before the expiration date as shown on the label under the condition of strictly avoiding light and storing at 2~8 °C ; The reagent is stable for 28 days after opening.

[References]

[1] Liu Limin,Hood Stefanie,Wang Yuping,Bezverkov Robert,Dou Chao,Datta Abhijit,Yuan Chong. Direct enzymatic assay for %HbAlc in human whole blood samples.[J]. Clinical Biochemistry,2008,41:576-583

[2] Teodoro-Morrison Tracy, Janssen Marcel J W et,al. Evaluation of a next generation direct whole blood enzymatic assay for hemoglobin Ale on the ARCHITECT c8000 chemistry system. [J].Clinical chemistry and laboratory medicine,2015,531

[3] Kozo Hirokawa,Kazuhiko Shimoji,Naoki Kajiyama. An enzymatic method for the determination of hemoglobinAlC[J]. Biotechnology Letters,2005,27 : 963-968.