Glycated albumin assay kit(GA-X)

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Manual Version: V1.0

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Catalog number

HOEM1005-01

HOEM1005-02

HOEM1005-03

HOEM1005-04

Applications[1,2]

Used for quantitative determination of glycated albumin (GA) and albumin (ALB) in human serum, the ratio of glycated albumin (%) is used glycated albumin concentration divided by the albumin concentration.

Glycated albumin is a non-enzymatic reaction between glucose and albumin in the blood, because the half-time of albumin in vivo is short (about 17-19 days), so GA serves as a 2-3 week indicator of average blood glucose. Therefore, it has significant clinical application value When blood sugar needs to be evaluated for short-term control.

Principle

1. Determination of glycated albumin

In the sample (serum), specific proteases are added to decompose Glycated albumin to form glycosylated amino acids. Then glycosylated amino acids are converted into glucoaldosterone, amino acids, and hydrogen peroxide by specific glycosylated amino acid oxidase. Under the action of peroxidase, hydrogen peroxide reacts with 4-amino-aminoantipyrine and TOOS to form red quinone-imine[3]. The concentration of glycated albumin in the sample was obtained by measuring its absorbance.

2. Determination of albumin

In pH 4.2, the albumin in the sample (serum) binds with the indicator bromocresol green to form a blue-green complex. The concentration of albumin in the sample was obtained by measuring the change of absorbance.

3. Calculation of glycated albumin value (%)

$$\text{Glycated albumin}(\%) = \frac{\text{GA（g/L）}}{\text{ALB（g/L）}} \times 100\%$$

The albumin reagent originated from Hzymes.

Reagents

Reagent	Components	Concentrations
GA	**Reagents 1（R1）：**	
	ADA buffer	20 mmol/L
	PRK	400 KU/L
	HTBA	10mmol/L
	Reagents 2（R2）：	
	FAOD	100KU/L
	Peroxidase	20KU/L
	4-Aminoantipyrine	10mmol/L
ALB	**Reagents 1（R1）**	
	Succinic acid buffer	120mmol/L
	Tween 80	0.1%
	Reagents 2（R2）	
	Succinic acid buffer	120mmol/L
	Bromocresol purple	0.15mmol/L

Sample requirements

1. Serum, heparin or EDTA plasma, and urine are suitable for samples. Whole blood, hemolysis is not recommended for use as a sample. Freshly drawn serum is the preferred specimen.

2. Stability: Serum/plasma: 7 days at 2-8℃;

Calibrator preparation

Carefully open the bottle, avoiding the loss of lyophilizate, and pipette in exactly 1.0 mL of distilled/deionized water. 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 distilled/deionized water. 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）GA：

Main wavelength	546nm	Subwavelength	700nm
Temperature	37℃	Type	2-End Point
Sample (calibration)		4μL	
R1		160μL	
Mix, incubated at 37 ℃ for 5min to determine the absorbance of A1.			
R2		40μL	
Mix, incubated at 37 ℃ for 5min to determine the absorbance of A2.			

$\Delta A = [(A2\text{-}A1)_{\text{sample}}]\text{-}[(A2\text{-}A1)_{\text{blank}}]$

2）ALB：

Main wavelength	600nm	Sub wavelength	660nm
Temperature	37℃	Type	2-End Point
Sample (calibration)		3μL	
R1		220μL	
Mix, incubated at 37℃ for 4.5min to determine the absorbance of A1.			
R2		110μL	
Mix, incubated at 37℃ for 9min to determine the absorbance of A2.			

$\Delta A = [(A2\text{-}A1)_{\text{sample}}]\text{-}[(A2\text{-}A1)_{\text{blank}}]$

Calibration

It is recommended to use the Calibrator from Hzymes and distilled/deionized water for

two-point calibration. If the reagent changes the batch, recalibration should be performed.

Quality control

At least two levels of control material should be analyzed with each batch of samples. Each laboratory should establish its own internal quality control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

Reference intervals[3]

Each laboratory should establish its own reference intervals based upon its patient population. The reference intervals measured at 37 °C listed below were taken from literature.
Serum / Plasma: 10.8%~17.1%

Interferences/specificity

The following substances were tested for interference with this methodology. Criterion: Recovery within ±10 % of initial value.

Substance	Level Tested	Observed Effect
Ascorbic acid	20 mg/dL	NSI*
Bilirubin	20 mg/dL	NSI
Lipemia	500 mg/dL	NSI
Hemoglobin	200 mg/dL	NSI
* NSI: No Significant Interference (within±10 %)		

Storage and stability

Up to the expiration date indicated on the label, when stored unopened at 2-8℃ and protected from light.
Once opened, the reagents are stable for 30 days when refrigerated on the analyzer or refrigerator.
Contamination of the reagents must be avoided.
Do not freeze the reagents.
Once dissolved, the calibrator is stable for 7 days at 2-8℃, the control is stable for 7 days at2-8℃, do not freeze.

Reagent blank absorbance

The Blank absorbance of the GA reagent should be ≤0.020; The Blank absorbance of the ALB reagent should be ≤0.35.

Analytical sensitivity

When the concentration of GA is 1.0 g/dL, the absorbance difference before and after the reaction should vary between 0.03 and 0.05;
When the concentration of ALB is 4.11 g/dL, the absorbance difference before and after the reaction should not be less than 0.2;

Precision

Within-run: CV≤5%
Between-run: CV≤10%

Linearity range

Conventional Units:
GA/ALB: 7.0%~70.0%
The concentration of the test sample should be within the linear range. If the value of sample exceeds 70.0%(GA/ALB), the sample should be diluted with 9 g/L NaCl solution.

Warnings and precautions

1.For in vitro diagnostic use.
2.Take the necessary precautions for the use of laboratory reagents.
3.Preservative contained. Do not swallow. Avoid contact with skin and mucous membranes.
4.Disposal of all waste material should be in accordance with local guidelines.
5.Material safety data sheet is available on request for professional users.

References

[1] Guide for clinical application of blood glucose monitoring in China (2011 edition). Chinese Journal of Diabetes, 2011, 3(1): 13 -21.
[2] Santiago Rodriguez-Segade et al. Progression of nephropathy in type 2 diabe -tes: The glycation gap is a significant predictor after adjustment for glycohemoglobin (HbA1c). Clinical Chemistry, 2011, 57(2):264-271.
[3] M. Koga, et al. Glycated albumin and glycated hemoglobin are influenced differently by endogenous insulin secretion in patients with type 2 diabetes. Diabetes care, 2010, 33(2): 270 – 272.