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CheKine™ Micro Glycosylated Serum Protein (GSP) Content Assay Kit

Cat #: KTB2226 Size: 48 T/48 S 96 T/96 S

	Micro Glycosylated Serum Protein (GSP) Content Assay Kit		
REF	**Cat #:** KTB2226	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum (plasma)		
	Storage: Stored at 4℃ for 24 months, protected from light		

Assay Principle

Glycated Serum Protein (GSP) refers to the substances in serum that, apart from hemoglobin, have amino groups at the N-terminus of albumin and other proteins which can undergo non-enzymatic glycation reactions with glucose to form ketoamine structures. It is similar to fructosamine. Clinically, GSP is detected to be used for diabetes diagnosis, treatment adjustment, and disease monitoring. The concentration of GSP reflects the situation of blood sugar in the past 1-3 weeks, while glycated hemoglobin (HbA1c) reflects the average blood sugar concentration over the past 9-12 weeks. In terms of reflecting the effect of blood sugar control, GSP appears earlier than HbA1c, and they are usually used together in clinical practice.

This kit uses the nitroblue tetrazolium (NBT) method. Under alkaline conditions, GSP in serum can reduce NBT to form a purple-red compound called formazan. This purple-red compound has a maximum absorption peak at 546 nm wavelength, and the rate of change in its absorbance is directly proportional to the concentration of GSP in the sample. Compared with the standard, the content of GSP in the sample is calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	10 mL	20 mL	4℃
Reagent Ⅱ	0.5 mL	0.5 mL	4℃
Reagent Ⅲ	2.5 mL	5 mL	4℃, protected from light
Standard	0.5 mL	0.5 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

·Microplate reader capable of measuring absorbance at 546 nm
·96-well plate, precision pipettes, disposable pipette tips
·Incubator, refrigerated centrifuge
·Deionized water

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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℃.

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

4 mmol/L Standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Note: Before opening the small-volume tubes, please centrifuge them at low speed first.

Sample Preparation

Note: It is recommended to use fresh samples. After blood collection, serum or plasma should be separated promptly to avoid hemolysis. Serum (plasma) samples should be tested as soon as possible after collection. If immediate testing is not possible, they should be aliquoted and stored at -80℃ for use within a month.

Serum (plasma) : Direct detection.

Assay Procedure

1. Preheat the microplate reader for more than 30 min, and adjust the wavelength to 546 nm.
2. Sample measurement. (The blank well, the standard blank well and the standard well only need to be tested once. Operate in 96-well plate as follows)

Reagent	Blank Well (μL)	Test Well (μL)	Standard Blank Well (μL)	Standard Well (μL)
Deionized water	10	0	0	0
Sample	0	10	0	0
Reagent Ⅱ	0	0	10	0
Standard	0	0	0	10
Reagent Ⅰ	160	160	160	160
Mix well, incubate at 37℃ for 5 min.				
Reagent Ⅲ	40	40	40	40

Mix well, incubate at 37℃ for 5 min. Read the absorbance value A_1 at 546 nm wavelength. Then incubate at 37℃ for 5 min and read the absorbance value A_2 at 546 nm wavelength. The absorbance value of the blank well is A_{Blank}, the absorbance value of the test well is A_{Test}, the absorbance value of the standard blank well is $A_{Standard\ Blank}$, and the absorbance value of the standard well is $A_{Standard}$. $\Delta A_{Blank}=A_{2Blank}-A_{1Blank}$, $\Delta A_{Standard\ Blank}=A_{2Standard\ Blank}-A_{1Standard\ Blank}$, $\Delta A_{Standard}=A_{2Standard}-A_{1Standard}$, $\Delta A_{Test}=A_{2Test}-A_{1Test}$.

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 the concentration:

GSP (mmol/L)=$(\Delta A_{Test}\div 5-\Delta A_{Blank}\div 5)\div(\Delta A_{Standard}\div 5-\Delta A_{Standard\ Blank}\div 5)\times C_{Standard}\times n=$**$(\Delta A_{Test}-\Delta A_{Blank})\div(\Delta A_{Standard}-\Delta A_{Standard\ Blank})\times 4\times n$**

Where: $C_{Standard}$: concentration of the standard , 4 mmol/L; 5: the interval for testing the values of A_1 and A_2 is 5 min; n: dilution factor.

Precautions

1. What if the obtained ΔA_{Test} is too high or too low?

If ΔA_{Test} is higher than 0.28, dilute the sample appropriately with deionized water and repeat the assay, multiply the result by the dilution factor. If ΔA_{Test} is lower than 0.03, increase the sample volume, then repeat the assay.

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2. Why set standard blank well?

Reagent Ⅱ is the matrix solution for dissolving the standards. It helps stabilize the standards but introduces background, so a standard blank well is included to subtract this background value.

Typical Data

Examples:

1. Test 10 μL bovine serum, following the above protocol and measured with the 96-well microplate:

$\Delta A_{Blank}=A_{2Blank}-A_{1Blank}=0.0443-0.0427=0.0016$, $\Delta A_{Standard\ Blank}=A_{2Standard\ Blank}-A_{1Standard\ Blank}=0.1110-0.0860=0.025$,

$\Delta A_{Standard}=A_{2Standard}-A_{1Standard}=0.3670-0.2330=0.134$, $\Delta A_{Test}=A_{2Test}-A_{1Test}=0.1710-0.1320=0.039$.

2. Calculation of the concentration:

$GSP\ (mmol/L)=(\Delta A_{Test}-\Delta A_{Blank})\div(\Delta A_{Standard}-\Delta A_{Standard\ Blank})\times4\times n=(0.039-0.0016)\div(0.134-0.025)\times4\times1=1.372$ mmol/L.

Recommended Products

Catalog No.	Product Name
KTB2225	CheKine™ Micro Glycosylated Hemoglobin (GHb) 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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