（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K069-M

Specification：48T(42 samples)/96T(90 samples)

Measuring instrument：Microplate reader（520-550 nm）

Detection range：0.06 - 4.0 mmol/L

Elabscience®Glycosylated Serum Protein (GSP) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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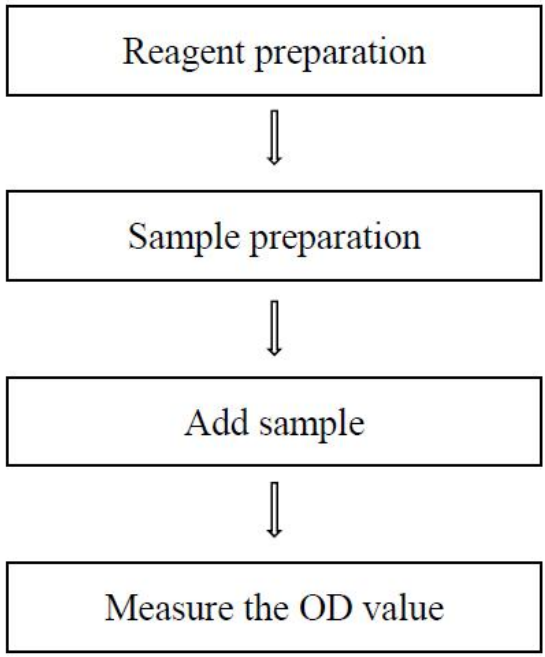
Assay summary



Intended use

This kit can measure glycosylated serum protein (GSP) content in serum and plasma samples.

Detection principle

Glycosylated serum protein (GSP), also called serum fructosamine, is formed by non-enzymatic reaction between glucose and serum protein in blood, and its content can effectively reflect the average blood glucose level in 1-3 weeks before measurement. Glycosylated serum protein is not affected by the temporary fluctuation of blood glucose concentration and provides a good indicator for the study of diabetes and long-term blood glucose level control. GSP with ketoamine structure is able to react with tetrazole blue under alkaline conditions to form purplish red product, which has a characteristic absorption peak at 530 nm, the concentration of GSP can be calculated by measuring the OD value.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Standard Diluent	0.5 mL ×1 vial	0.5 mL × 1 vial	-20℃, 12months
Reagent 2	2 mmol/L Standard Solution	0.5 mL ×1 vial	0.5 mL × 1 vial	-20℃, 12months
Reagent 3	Chromogenic Agent	12 mL × 1 vial	25 mL × 1 vial	-20℃, 12 months, shading light
Reagent 4	Stop Solution	3 mL × 1 vial	6 mL × 1 vial	-20℃, 12months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Incubator, Microplate reader (520-550 nm, optimum wavelength: 530 nm)

Reagent preparation

Preheat chromogenic agent at 37°C for 1 hour before use. Equilibrate other reagents to room temperature before use.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Rat serum	1
Mouse serum	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① Avoid bubbles when adding chromogenic agent.

② Avoid using chylous and hemolysis samples, which can interfere with the results.

③ Preheat chromogenic agent at 37°C before adding to the chromogenic system.

Operating steps

① Standard$_{(control)}$ well: Add 10 μL of standard diluent.

Standard well: Add 10 μL of 2 mmol/L standard solution.

Blank well: Add 10 μL of double distilled water.

Sample well: Add 10 μL of sample.

② Add 200 μL of chromogenic agent to each well.

③ Mix fully and incubate at 37℃ for 15 min.

④ Add 50 μL of stop solution to each well.

⑤ Mix fully, measure the OD value of each well at 530 nm with microplate reader.

Calculation

The sample:

1. Serum (plasma) sample:

$$\text{GSP content (mmol/L)} = (A - A_0) \div (A_2 - A_1) \times 2^*$$

[Note]

A: The OD value of sample well.

A_0: The OD value of blank well.

A_2: The OD value of standard well.

A_1: The OD value of standard(control) well.

2*: The concentration of standard, 2 mmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.85	2.30	3.40
%CV	4.6	4.0	4.6

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.85	2.30	3.40
%CV	6.5	6.1	6.3

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 104%.

	Sample 1	Sample 2	Sample 3
Expected Conc. （mmol/L）	1.2	2.7	3.6
Observed Conc. （mmol/L）	1.2	2.8	3.8
recovery rate(%)	102	105	105

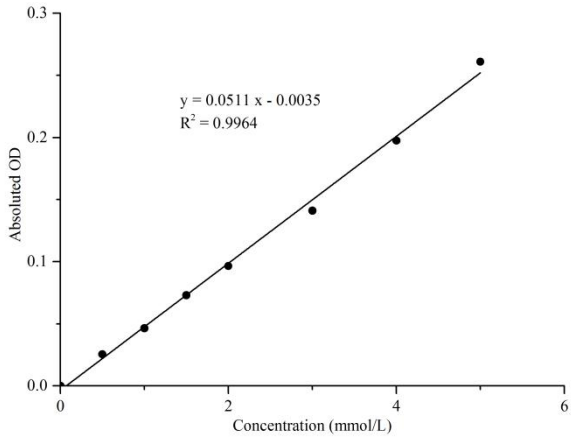
Sensitivity

The analytical sensitivity of the assay is 0.06 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	**0**	**0.5**	**1**	**1.5**	**2**	**3**	**4**	**5**
OD value	0.076	0.099	0.124	0.152	0.170	0.224	0.280	0.339
	0.076	0.104	0.121	0.146	0.175	0.210	0.267	0.335
Average OD	0.076	0.102	0.123	0.149	0.173	0.217	0.274	0.337
Absoluted OD	0.000	0.026	0.047	0.073	0.097	0.141	0.198	0.261



Appendix II Example Analysis

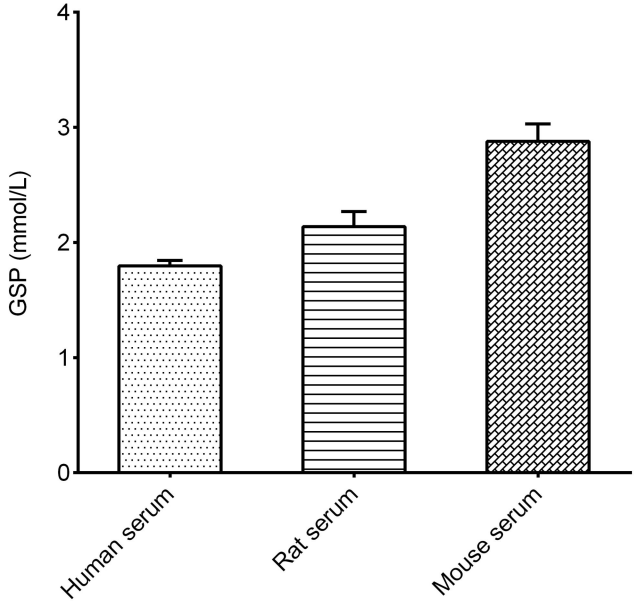
Example analysis:

For human serum, carry the assay according to the operation table. The results are as follows:

The average OD value of the standard$_{(control)}$ is 0.111, the average OD value of standard is 0.255, the average OD value of the blank is 0.044, the average OD value of the sample is 0.174, and the calculation result is:

$$\text{GSP content (mmol/L)} = (0.174 - 0.044) \div (0.255 - 0.111) \times 2 = 1.81 \text{ mmol/L}$$

Detect human serum, rat serum and mouse serum according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.