PRODUCT INFORMATION & MANUAL

Glycosylated Serum Protein/GSP Assay Kit (Colorimetric)

NBP3-25813

For research use only.
Not for diagnostic or therapeutic procedures.

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Glycosylated Serum Protein/GSP Assay Kit (Colorimetric)

Catalog No: NBP3-25813

Method: Colorimetric method

Specification: 96T (Can detect 90 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.06 mmol/L

Detection range: 0.06 - 4.0 mmol/L

Average intra-assay CV (%): 4.4

Average inter-assay CV (%): 6.3

Average recovery rate (%): 104

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲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	Specification	Storage
Reagent 1	Standard Diluent	0.5 mL × 1 vial	-20℃ , 12 months
Reagent 2	2 mmol/L Standard Solution	0.5 mL × 1 vial	-20℃ , 12 months
Reagent 3	Chromogenic Agent	25 mL × 1 vial	-20℃ , 12months, shading light
Reagent 4	Stop Solution	6 mL×1 vial	-20℃ , 12 months
	Microplate	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.			

▲Materials prepared by users

Instruments

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

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. Avoid bubbles when adding regent 3.
2. Avoid using chylous and hemolysis samples, which can interfere with the results.
3. Preheate reagent 3 at 37°C before adding to the chromogenic system.

Pre-assay preparation

▲ Reagent preparation

Preheat reagent 3 at 37°C for 1 hour and bring other reagents to room temperature before use.

▲ Sample preparation

Serum and plasma samples:

Detect the sample directly. If the sample is turbidity, centrifuge at 4°C at 5000 g for 5 min, then take the supernatant for detection.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.06 - 4.0 mmol/L).

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).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83
B	B	B	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84
C	X	X	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
D	S1	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
E	S2	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
F	S3	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
G	S4	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
H	S5	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90

Note: A, standard$_{(control)}$ wells; B, standard wells; X, blank wells; S1-S90, sample wells.

▲Detailed operation steps

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

Standard well: Add 10 μL of regent 2.

Blank well: Add 10 μL of double distilled water.

Sample well: Add 10 μL of sample.

(2) Add 200 μL of regent 3 to each well.

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

(4) Add 50 μL of regent 4 to each well.

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

▲Summary operation table

	Standard$_{control}$ well	Standard well	Blank well	Sample well
Regent 1 (μL)	10			
Regent 2 (μL)		10		
Double distilled water (μL)			10	
Sample (μL)				10
Regent 3 (μL)	200	200	200	200
Mix fully and incubate at 37℃ for 15 min.				
Regent 4 (μL)	50	50	50	50
Mix fully, measure the OD value of each well.				

▲Calculation

1. Serum/plasma :

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

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 $\text{standard}_{(\text{control})}$ well.

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

Appendix Data

▲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:

GSP content (mmol/L) = (0.174 – 0.044) ÷ (0.255 – 0.111) × 2 = 1.81 mmol/L

Appendix II References

1. Xu Y.J. A Convenient Assay of Glycoserum by Nitroblue Tetrazolium with Indoacetamide. Clinica Chimica Acta, 325(2002): 127-131.
2. Abidin D. An Improved Enzymatic Assay for Glycated Serum Protein. Analytic Method, 2013, 5: 2461-2469.