TECHNICAL MANUAL

Glycosylated Serum Protein (GSP) Colorimetric Assay Kit

- **SKU CODE:** MAES0222
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Sample preparation
- Reagent preparation
- Add standards and samples
- Incubation at 37°C
- Add stop solution
- Measure OD at 530 nm

2. Intended use

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

3. 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 a purplish red product, which has a characteristic absorption peak at 530 nm. The concentration of GSP can be calculated by measuring the OD value.

4. 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°C, 12 months
Reagent 2	2 mmol/L Standard Solution	0.5 mL × 1 vial	0.5 mL × 1 vial	-20°C, 12 months
Reagent 3	Chromogenic Agent	12 mL × 1 vial	25 mL × 1 vial	-20°C, 12 months, protect from light
Reagent 4	Stop Solution	3 mL × 1 vial	6 mL × 1 vial	-20°C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

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

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 small volume reagents, please centrifuge before use to ensure sufficient amount of reagents can be obtained.

6. Reagent preparation

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

7. 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°C for a month.
2. **Dilution of sample:** The recommended dilution factor for different samples is as follows (for reference only): 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 perform a pretest to confirm the dilution factor.

8. 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.

9. 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°C 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.

10. Calculation

The sample:

1. Serum (plasma) sample:

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.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision Results

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.

Inter-assay Precision Results

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

Recovery

Three samples of high concentration, middle concentration and low concentration were tested 6 times in parallel for each concentration to obtain an average recovery rate of 104%.

Recovery Results

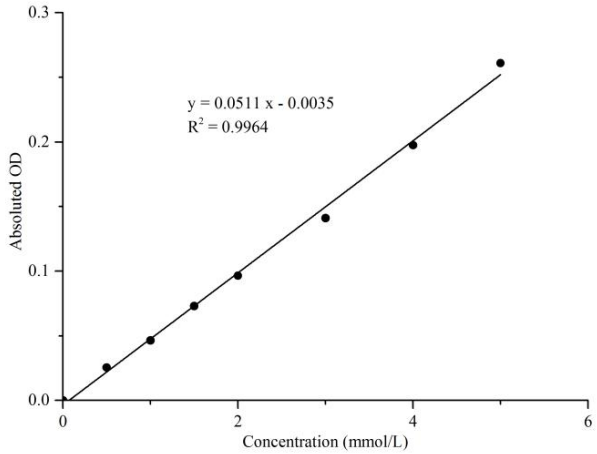
Sample	Expected Conc. (mmol/L)	Observed Conc. (mmol/L)	Recovery rate(%)
Sample 1	1.2	1.2	102
Sample 2	2.7	2.8	105
Sample 3	3.6	3.8	105

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.

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), the standard curve and data are provided below for reference only:



Standard curve data

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

12. Appendix II Example Analysis

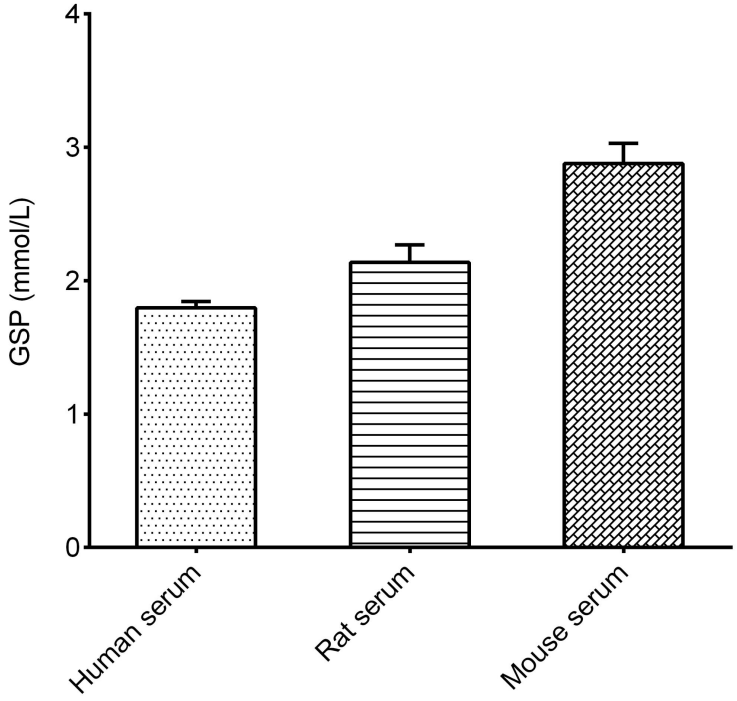
Example analysis:

For human serum, carry out 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

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



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance is not within the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to calculate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.