# Glycosylated Serum Protein (GSP) Content Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer

**Catalog Number:** BC4940

**Size**: 50T/48S

**Components:**

| Reagent | Size | Storage |
|---|---|---|
| Reagent I | Solution 5 mL×1 | 4℃ |
| Reagent II | Solution 2 mL×1 | 4℃ |
| Reagent III | Solution 60 mL×1 | 4℃ |
| Reagent IV | Solution 5 mL×1 | 4℃ |
| Standard | Powder×1 | 4℃ |

Solution preparation:

Standard solution: Add 0.8 mL Reagent II to prepare the 10 mmol/L DMF standard solution before use. Dilute the 10 mmol/L standard solution into the 4 mmol/L standard solution with Reagent II for test.

**Product Description：**

Serum glucose reacts non-enzymatically with the N-terminal amino group of albumin and other serum protein molecules to form a polymer ketamine structure. Under alkaline conditions, it reacts with nitrotetrachloroazole blue to produce formazan, a purple-red compound. Comparing the color at 530 nm wavelength, measuring its OD value. And comparing with DMF standard, the content can be obtained.

**Reagents and Equipment Required but Not Provided：**

Spectrophotometer, desk centrifuge, transferpettor, 1 mL glass cuvette, mortar/homogeniser, ice and distilled water.

**Procedure**

**I. Sample preparation:**

Serum (plasma): collecting bacteria or cells into the centrifuge tube. According to the ratio of Serum (plasma)volume (mL): Reagent I volume (mL) = 10:1. It is suggested to take about 0.5 mL serum (plasma) and add 0.05 mL of Reagent I. Mix thoroughly. Stay in 37℃ for 30 min.

**II. Determination procedure：**

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 530 nm, set zero with distilled water.
2. Standard solution: According to the ratio of Standard solution volume (mL): Reagent I volume (mL) = 10:1. It is suggested to take about 0.5 mL Standard solution and add 0.05 mL of Reagent I. Mix thoroughly. Stay in 37℃ for 30 min.

3. Add reagents with the following list:

| Reagent（μL） | Test tube | Blank tube | Standard tube | Standard blank tube |
|---|---|---|---|---|
| Sample | 50 | - | - | - |
| Distilled water | - | 50 | - | - |
| Standard | - | - | 50 | - |
| Reagent II | - | - | - | 50 |
| Reagent III | 1000 | 1000 | 1000 | 1000 |
| Stay in 37℃ for15 min； | | | | |
| Reagent IV | 50 | 50 | 50 | 50 |
| Mix thoroughly. Take 1 mL to 1 mL glass cuvette. Measure the absorbance at 530 nm. Record as $A_T$、$A_B$、$A_S$、$A_{SB}$。$\Delta A_T=A_T-A_B$，$\Delta A_S=A_S-A_{SB}$。 | | | | |

Note: Blank tube and Standard tube only need to test one~two times.

**III. Calculations：**

$GSP(mmol/L) =C\times\Delta A_T\div\Delta A_S\times F$

C: Concentration of Standard solution, 4 mmol/L;

F: Dilution times.

**Note:**

1. After color development, please add Reagent IV immediately. It is recommended not to make too many samples at once.
2. If the measured absorbance value A>1.0 or ΔA>0.8, it is recommended to dilute the sample before measuring, and multiply the dilution factor in the calculation formula; if the measured absorbance value is low or close to the blank OD value, it is recommended to increase the sample volume before performing the measurement.

**Experimental example**

1. Take 0.5 mL mouse plasma and standard solution. Add 0.05 mL of Reagent I. Mix thoroughly. Stay in 37℃ for 30 min. Follow the measurement procedure. Calculate $\Delta A_T=A_T-A_C=0.315-0.002=0.313$. $\Delta A_S=A_S-A_{SB}=0.29-0.092=0.198$. Calculate the content of glycated serum protein in mouse plasma according to the formula:
   $GSP (mmol/L) =C\times\Delta A_T\div\Delta A_S=4\times0.313\div0.198=6.323$ mmol/L
2. Take 0.5 mL horse serum and standard solution. Add 0.05 mL of Reagent I. Mix thoroughly. Stay in 37℃ for 30 min. Follow the measurement procedure. Calculate $\Delta A_T=A_T-A_C=0.098-0.002=0.096$. $\Delta A_S=A_S-A_{SB}=0.29-0.092=0.198$. Calculate the content of glycated serum protein in mouse plasma according to the formula:
   $GSP (mmol/L) =C\times\Delta A_T\div\Delta A_S=4\times0.096\div0.1989=1.939$ mmol/L