

Instructions for Use

Version: 1.0.1
Revision date: 25-Sep-23

Glycosylated Serum Protein (GSP) Assay Kit

Catalog No.: abx298947

Size: 96 tests

Detection Range: 0.06 mmol/L – 4.0 mmol/L

Sensitivity: 0.06 mmol/L

Storage: Store all components at -20°C in the dark.

Application: For detection and quantification of Glycosylated Serum Protein in serum and plasma samples.

Introduction

Abbexa's Glycosylated Serum Protein (GSP) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Glycosylated Serum Protein (GSP) concentration. GSP present in samples reacts with tetrazole blue under alkaline conditions to produce a compound that has an absorption maximum at 530 nm. The intensity of yellow color is proportional to the concentration of GSP, which can then be calculated.

Kit components

1. 96-well microplate
2. Standard Diluent: 0.5 ml
3. Detection Reagent: 25 ml
4. Standard (2 mmol/L): 0.5 ml
5. Stop Solution: 6 ml
6. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (530 nm)
2. Double distilled water
3. Normal Saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Incubator
7. Vortex mixer

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Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Collect the serum using a serum separator tube and allow to stand for 1-2 h at room temperature or overnight at 4°C. Centrifuge for 15 min at 2000 × g at 4°C. Transfer the supernatant into a clean tube and analyse immediately. Bring samples to room temperature before carrying out the assay.
- **Plasma:** Collect the plasma in a tube using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at 1000 × g at 2-8°C within 30 min of collection. Transfer the supernatant into a clean tube and analyse immediately. Bring samples to room temperature before carrying out the assay.

Notes:

- Fresh samples or recently obtained samples are recommended to prevent degradation that may lead to erroneous results.
- Samples should be free of hemolysis or turbidity.
- Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Normal Saline (0.9% NaCl) then carry out the assay procedure.

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

2. Reagents

Bring all reagents to room temperature prior to use.

- **Detection Reagent:** Preheat at 37°C for one hour before use.

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B. Assay Procedure

1. Set Standard, Standard Control, Blank and Sample wells on the 96 well microplate and label accordingly. It is recommended to test Standard, Standard Control and Blank in duplicate.
2. Add 10 µl of standard diluent to the standard control wells.
3. Add 10 µl of Standard (2 mmol/L) to the standard wells.
4. Add 10 µl of double distilled water to the blank wells.
5. Add 10 µl of sample to the blank wells.
6. Add 200 µl of Detection Reagent (preheated to 37°C) to the all wells.
7. Mix fully, and incubate at 37°C for 15 minutes.
8. Add 50 µl of Stop Solution to all wells and mix fully.
9. Read and record the absorbance at 530 nm with a microplate reader.

C. Calculation of Results

$$\text{GSP (mmol/L)} = \frac{A - A_0}{A_2 - A_1} \times 2$$

where:

A	OD value of the sample
A ₀	OD value of the blank
A ₂	OD value of the standard
A ₁	OD value of the standard control
2	Concentration of the standard (2 mmol/L)