Glycated Serum Protein Microplate Assay Kit User Manual

Catalog # FTA0172

(Version 1.2A)

Detection and Quantification of Glycated Serum Protein (GSP) Content in Serum, Plasma, Cell culture, Urine, Other biological fluids Samples.

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

I. INTRODUCTION

Glycated serum proteins (GSP; glycated albumins; fructosamine) are a medium term indicator of diabetic control (2-3 weeks). Fructosamines are compounds that result from glycation reactions between a sugar (such as fructose or glucose) and a primary amine, followed by isomerization via the Amadori rearrangement. Biologically, fructosamines are recognized by fructosamine-3-kinase, which may trigger the degradation of advanced glycation end-products.

Glycated Serum Protein Microplate Assay Kit is based on the NBT-based methods to test fructosamine content. The increase in absorbance at 530 nm after addition of the stop reagent is directly proportional to the glycated serum proteins content.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Stop Solution	2 ml x 1	4 °C
Dye Reagent	Powder x 1	-20 °C
Standard	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Dye Reagent: add 6 ml Reaction Buffer to dissolve before use.

Standard: add 0.4 ml distilled water to dissolve before use, mix, the concentration will be 10 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 530 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For liquid samples

For serum and plasma samples, detect directly.

For urine samples containing precipitation, centrifuge at 10,000 x g, 4°C for 3 minutes and assay the supernatant.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Reaction Buffer	110 µl	110 µl	110 µl
Mix, put it in the oven, 37 °C for 10 minutes.			
Dye Reagent	60 µl	60 µl	60 µl
Mix, put it in the oven, 37 °C for 15 minutes.			
Stop Solution	20 µl	20 µl	20 µl
Mix, record absorbance measured at 530 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

VI. CALCULATION

1. According to the protein concentration of sample

$$GSP\ (\mu mol/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (C_{Protein} \times V_{Sample})$$

$$= 10 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$GSP\ (\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$$

$$= 10 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of sample

$$GSP\ (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 10 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the concentration of Standard, 10 mmol/L = 10 μmol/ml;

W: the weight of sample, g;

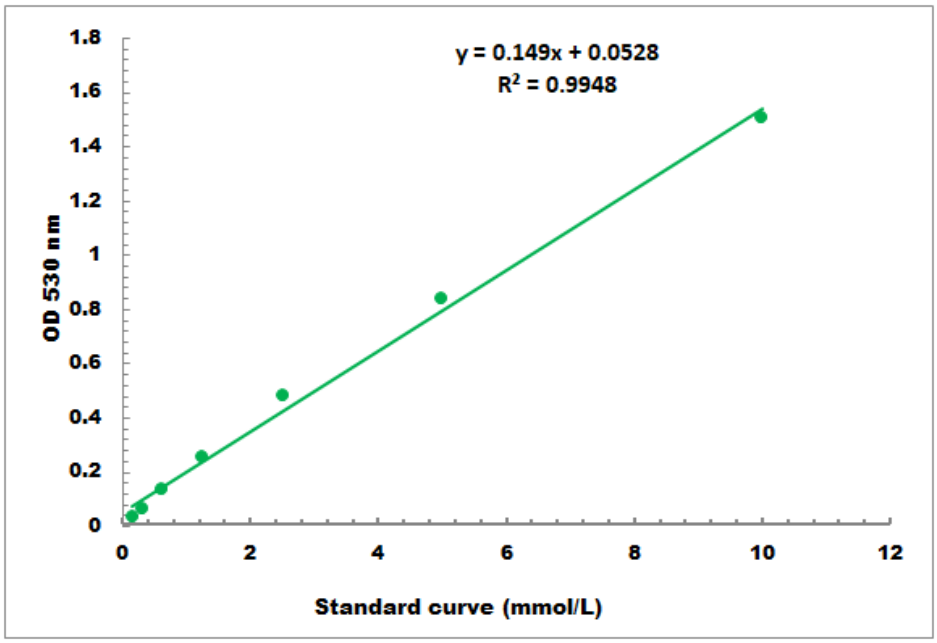
$V_{Standard}$: the volume of standard, 0.01 ml;

V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 mmol/L - 10 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES