



Glycogen Synthase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1168

(Version 1.4B)

Detection and Quantification of Glycogen Synthase (GCS)

Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture
media and Other biological fluids Samples.

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

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I. INTRODUCTION

Glycogen synthase (EC 2.4.1.11) is a key enzyme in glycogenesis, the conversion of glucose into glycogen. It is a glycosyltransferase that catalyses the reaction of UDP-glucose and $(1,4\text{-}\alpha\text{-D-glucosyl})_n$ to yield UDP and $(1,4\text{-}\alpha\text{-D-glucosyl})_{n+1}$. In other words, this enzyme combines excess glucose residues one by one into a polymeric chain for storage as glycogen. Glycogen synthase concentration is highest in the bloodstream 30 to 60 minutes following intense exercise.

Glycogen Synthase Activity Colorimetric Microplate Assay Kit is a sensitive assay for determining Glycogen synthase activity in various samples. Glycogen synthase activity is determined by NADH decomposition rate. The reaction products can be measured at a colorimetric readout at 340 nm.

II. KIT COMPONENTS

| Component | Volume | Storage |
|--------------------|------------|---------|
| 96-Well Microplate | 1 plate | |
| Assay Buffer | 30 mlx 4 | 4 °C |
| Reaction Buffer I | 5 mlx 1 | 4 °C |
| Substrate | Powder x 1 | -20 °C |
| Reaction Buffer II | 15mlx 1 | 4 °C |
| Enzyme | Powder x 1 | -20 °C |
| Standard | Powder x 1 | -20 °C |
| Technical Manual | 1 Manual | |

Note:

Substrate: add 1 ml Reaction Buffer I to dissolve before use.

Enzyme: add 1 ml Reaction Buffer II to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 $\mu\text{mol/L}$.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10,000g 4°C for 15 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Warm all reagents to 37 °C before use.

Add following reagents into the microplate:

| Reagent | Sample | Standard | Blank |
|---|--------|----------|--------|
| Reaction Buffer I | 40 µl | -- | -- |
| Substrate | 10 µl | -- | -- |
| Sample | 10 µl | -- | -- |
| Mix, incubate at 37 °C for 5 mins. | | | |
| Standard | -- | 200 µl | -- |
| Distilled water | -- | -- | 200 µl |
| Reaction Buffer II | 130 µl | -- | -- |
| Enzyme | 10 µl | -- | -- |
| Mix, measured at 340 nm and record the absorbance of 10th second and 70th second. | | | |

Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- 3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One Unit of Glycogen Synthase activity is defined as the enzyme reduces 1 μmol of NADH per minute.

1. According to the protein concentration of sample

$$\begin{aligned} \text{GCS (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(70\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 1.6 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(70\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{GCS (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(70\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times W / V_{\text{Assay}}) / T \\ &= 1.6 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(70\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{GCS (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(70\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times N / V_{\text{Assay}}) / T \\ &= 1.6 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(70\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

4. According to the volume of sample

$$\begin{aligned} \text{GCS (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(70\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad V_{\text{Sample}} / T \\ &= 1.6 \times (\text{OD}_{\text{Sample}(10\text{S})} - \text{OD}_{\text{Sample}(70\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

C_{Standard} : the standard concentration, 400 $\mu\text{mol/L}$ = 0.4 $\mu\text{mol/ml}$;

V_{Standard} : the volume of standard, 200 μl = 0.2 ml;

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

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

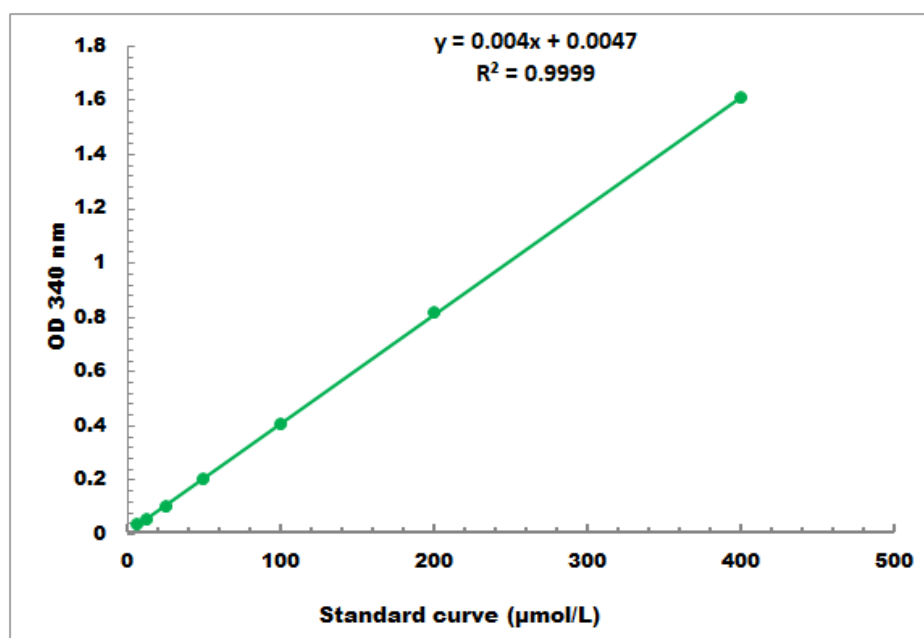
V_{Sample} : the volume of sample, 0.01 ml;

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

T: the reaction time in step 1, 5 minutes.

VII. TYPICAL DATA

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



Detection Range: 4μmol/L - 400 μmol/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