



Glycogen Microplate Assay Kit

User Manual

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Detection and Quantification of Glycogen Content in 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 is a multibranched polysaccharide of glucose that serves as a form of energy storage in humans, animals, and fungi. The polysaccharide structure represents the main storage form of glucose in the body.

In humans, glycogen is made and stored primarily in the cells of the liver and the muscles, hydrated with three or four parts of water. Glycogen functions as the secondary long-term energy storage, with the primary energy stores being fats held in adipose tissue. Muscle glycogen is converted into glucose by muscle cells, and liver glycogen converts to glucose for use throughout the body including the central nervous system.

Glycogen Microplate Assay Kit is a sensitive assay for determining Glycogen in various samples. Glycogen concentration is determined by anthrone. The reaction products can be measured at a colorimetric readout at 620 nm.



II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Dye Reagent	Powder x 1	4 °C
Dye Reagent Diluent	15 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

Standard: add 1 ml distilled water to dissolve before use, then add 20 µl into 980 µl distilled water, the concentration will be 200 µg/ml.

Dye Reagent: add 15 ml Dye Reagent Diluent to dissolve before use, store at 4 °C.

Dye Reagent Diluent: be careful, it is strong acid.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 620 nm
2. Distilled water
3. 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%, sonication 3s, interval 10s, repeat 30 times); centrifuged at 10,000g 4 °C for 10 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

Add 0.9 ml Assay buffer into 0.1 ml liquid sample, centrifuged at 10,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.



V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	50 µl	--	--
Standard	--	50 µl	--
Distilled water	--	--	50 µl
Dye Reagent	150 µl	150 µl	150 µl

Mix, put the microplate into the oven, incubate at 90 °C for 15 minutes, when cold record absorbance measured at 620 nm.

VI. CALCULATION

1. According to the protein concentration of sample

$$\begin{aligned}\text{Glycogen } (\mu\text{g}/\text{mg}) &= 1.11 \times (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \\ &\quad \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times C_{\text{Protein}}) \\ &= 222 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}}\end{aligned}$$

2. According to the weight of sample

$$\begin{aligned}\text{Glycogen } (\mu\text{g}/\text{g}) &= 1.11 \times (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \\ &\quad \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times W / V_{\text{Assay}}) \\ &= 222 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W\end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned}\text{Glycogen } (\mu\text{g}/10^4) &= 1.11 \times (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \\ &\quad \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times N / V_{\text{Assay}}) \\ &= 222 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N\end{aligned}$$

4. According to the volume of sample

$$\begin{aligned}\text{Glycogen } (\mu\text{g}/\text{ml}) &= 1.11 \times (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \\ &\quad \text{OD}_{\text{Blank}}) / V_{\text{Sample}} \\ &= 222 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})\end{aligned}$$

1.11: the constant for converting glucose content into glycogen content;

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

C_{Standard} : the protein concentration, 200 $\mu\text{g}/\text{ml}$;

W: the weight of sample, g;

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

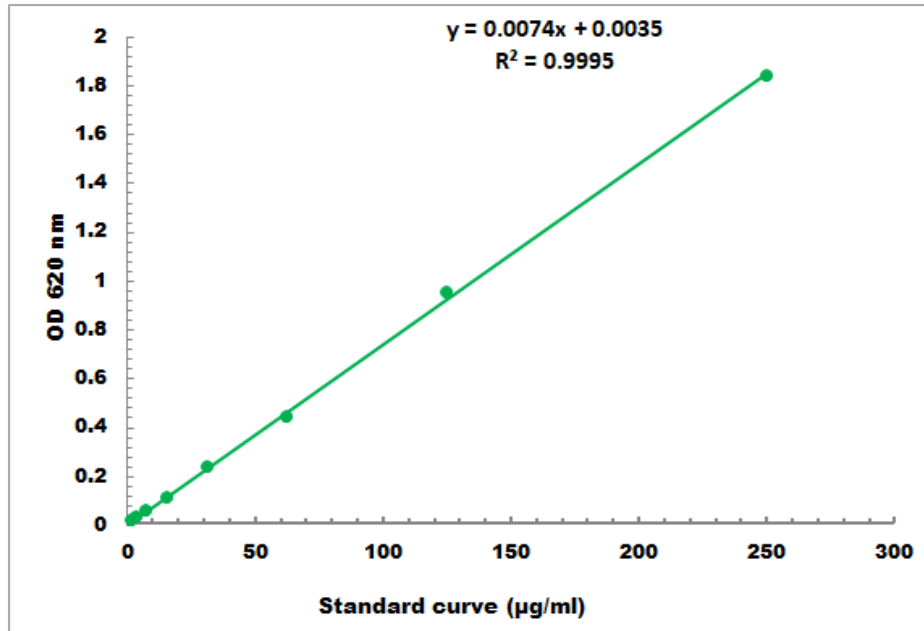
V_{Standard} : the volume of standard, 50 μl ;

V_{Sample} : the volume of sample, 50 μl .

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: 2 µg/ml - 200 µg/ml