Glycogen Microplate Assay Kit

Cat #: orb545617 (manual)

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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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. In the assay, glucoamylase hydrolyzes the glycogen to glucose which is then specifically oxidized to produce a product that reacts with dye reagent to generate color. The reaction products can be measured at a colorimetric readout at 505 nm.

KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	--
Assay Buffer	30 ml x 4	4 °C
Enzyme	Powder x 1	-20 °C
Reaction Buffer	5 ml x 1	4 °C
Dye Reagent A	Powder x 1	-20 °C
Dye Reagent B	Powder x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	--
Technical Manual	1 Manual	

Note:

Enzyme: add 1 ml Reaction Buffer to dissolve before use, store at 4 °C.

Dye Reagent A: add 7 ml distilled water to dissolve before use.

Dye Reagent B: add 7 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use, mix, the concentration will be 2 mg/ml.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 505 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

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) ; boil the lysates for 10 minutes, centrifuged at 10, 000g 4 °C for 10 minutes, transfer the supernatant into a new centrifuge tube for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer; boil the homogenates for 10 minutes; centrifuged at 10, 000g for 10 minutes, transfer the supernatant into a new centrifuge tube for detection.

3. For liquid samples

Liquid samples can be assayed directly.

ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	40 µl	50 µl	50 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Enzyme	10 µl	--	--
Distilled water	--	--	10 µl
Mix, put the plate into the oven, 50 °C for 60 minutes.			
Dye Reagent A	70 µl	70 µl	70 µl
Dye Reagent B	70 µl	70 µl	70 µl
Mix, put the plate into the oven, 37 °C for 15 minutes, record absorbance measured at 505 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.

CALCULATION

1. According to the weight of sample

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

$$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

2. According to the quantity of cells or bacteria

$$\text{Glycogen } (\mu g/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay})$$

$$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

3. According to the volume of sample

$$\text{Glycogen } (\mu g/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

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

$C_{Standard}$: the protein concentration, 2 mg/ml;

W: the weight of sample, g;

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

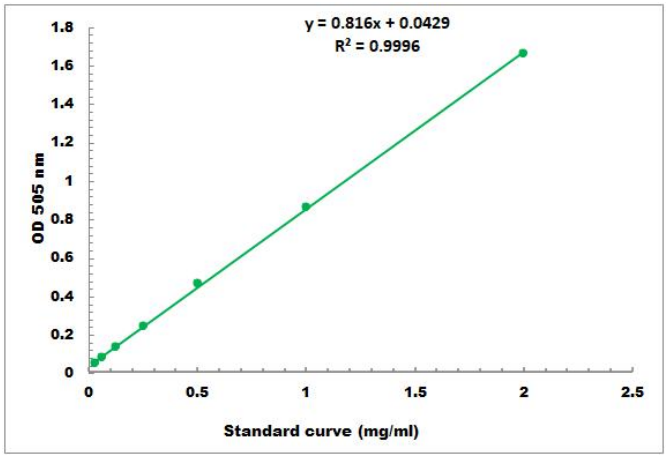
$V_{Standard}$: the volume of standard, 10 µl;

V_{Sample}: the volume of sample, 10 µl;

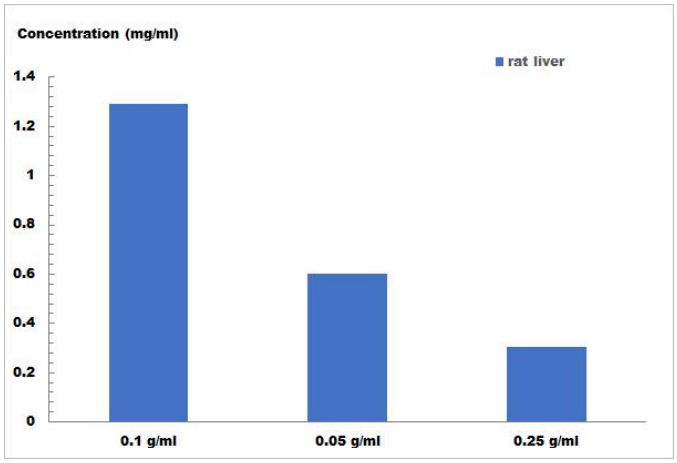
V_{Assay}: the volume of Assay buffer, 1 ml.

TYPICAL DATA

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



Detection Range: 0.02 mg/ml - 2 mg/ml



Rat liver samples were assayed according to the kit protocol.