Glycogen

Fluorometric Microplate Assay Kit

User Manual

Catalog # CAK8015

(Version 1.1A)

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.

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 Fluorometric 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 compound that reacts with the probe, which can be detected fluorometrically (Ex/Em 535/587).

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
Enzyme	Powder x 1	-20 °C
Probe	Powder x 1	-20 °C, keep in dark
Probe Diluent	1 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	--
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Note:

Enzyme: add 5 ml Reaction Buffer to dissolve before use. Aliquot & store at -20 °C. Use within one month.

Probe: Warm Probe Diluent to RT prior to use to melt frozen Probe Diluent; then add 1 ml Probe Diluent to dissolve. Store at -20 °C, protect from light and moisture. Use within one month.

Standard: add 1 ml distilled water to dissolve before use, mix; then add 50 µl into 950 µl Assay Buffer, the concentration will be 0.1 mg/ml. Store at -20 °C. Use within one month.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Fluorescence microplate reader to read fluorescence at Ex/Em = 535/587
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

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); 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

Detect directly or dilute with Assay Buffer.

V. ASSAY PROCEDURE

Warm the solution to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	170 µl	170 µl	170 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Probe	10 µl	10 µl	10 µl
Enzyme	10 µl	10 µl	10 µl
Mix, put it in the oven, 37 °C for 10 minutes, protected from light, record fluorescence measured at Ex/Em = 535/587 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the weight of sample

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

$$= 100 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

2. According to the quantity of cells or bacteria

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

$$= 100 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

3. According to the volume of sample

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

$$= 100 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

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

$C_{Standard}$: the protein concentration, 0.1 mg/ml = 100 μg/ml

W: the weight of sample, g

N: the quantity of cell or bacteria, N × 10^4

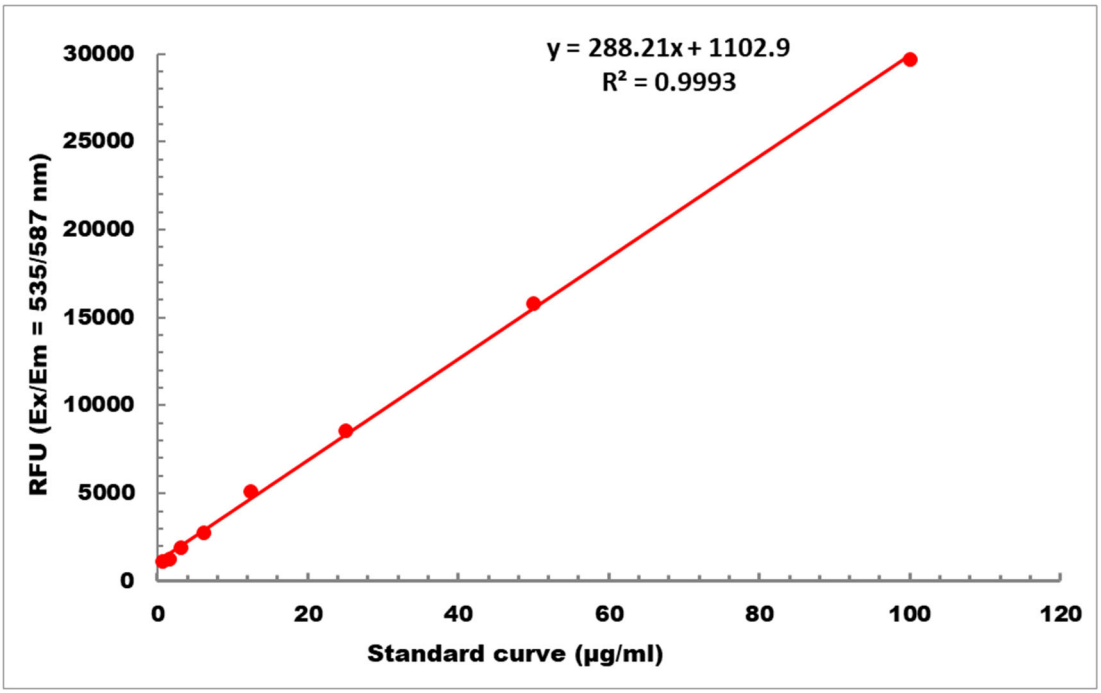
$V_{Standard}$: the volume of standard, 10 μl

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

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

VII. TYPICAL DATA

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



Detection Range: 1 µg/ml - 100 µg/ml

VIII. TECHNICAL SUPPORT

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

IX. NOTES