Product Manual

Glycogen Assay Kit (Fluorometric)

Catalog Number

MET-5023 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Glycogen is a polysaccharide found in animals as well as simpler organisms such as fungi. Glycogen is made up of glucose monomers and is considered the primary method of storing glucose in animals. In humans, glycogen is mainly synthesized in the liver (up to 6% of the total mass) and the muscles (approximately 2% of the total mass). Lesser amounts of glycogen are found in the kidneys, glial cells in the brain, and white blood cells. Glycogen also serves as an energy source in the uterus during pregnancy to supply glucose to the fetus. While fats in adipose tissue represent the main stored energy source, glycogen is the second most abundant stored energy source. Glycogen is similar in structure and function to starch, a polysaccharide composed of glucose monomers that also serves as energy storage in plants. Glycogen differs structurally from starch in that it is more extensively branched and compact than starch.

Glycogen storage diseases (GSD) are a set of disorders caused by the disruption of glycogen metabolism. Disruption of the liver's ability to supply the rest of the body with glycogen can lead to low blood sugar levels. Additionally, if liver glycogen is not broken down effectively, this can lead to abnormal enlargement of the liver. Glycogen synthase mutations causing impaired protein function causes lower glycogen synthesis and causes the liver to be small. Finally, glycogen brancher deficiency can cause aberrant forms of glycogen to be stored in the liver ultimately causing a disorder of progressive liver dysfunction.

Cell Biolabs' Glycogen Assay Kit is a simple fluorometric assay that measures the amount of total glycogen present in biological samples in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays*, including blanks, glycogen standards, and unknown samples. Sample glycogen concentrations are determined by comparison with a known glycogen standard. The kit has a detection sensitivity limit of 120 nM glycogen.

Note: Each sample replicate requires 2 assays, one treated with amyloglucosidase (+AG) and one without (-AG). Glycogen is calculated from the difference in RFU readings from the 2 wells.

Assay Principle

Cell Biolabs' Glycogen Assay Kit measures total glycogen within biological samples. Glycogen is broken down into glucose monomers by amyloglucosidase first, glucose is then oxidized by glucose oxidase into D-gluconic acid and hydrogen peroxide. The resulting hydrogen peroxide is then detected with a highly specific fluorometric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples are compared to a known concentration of glycogen standard within the 96-well microtiter plate format. Samples and standards are incubated for 45 minutes and then read with a standard 96-well fluorometric plate reader (Figure 1).

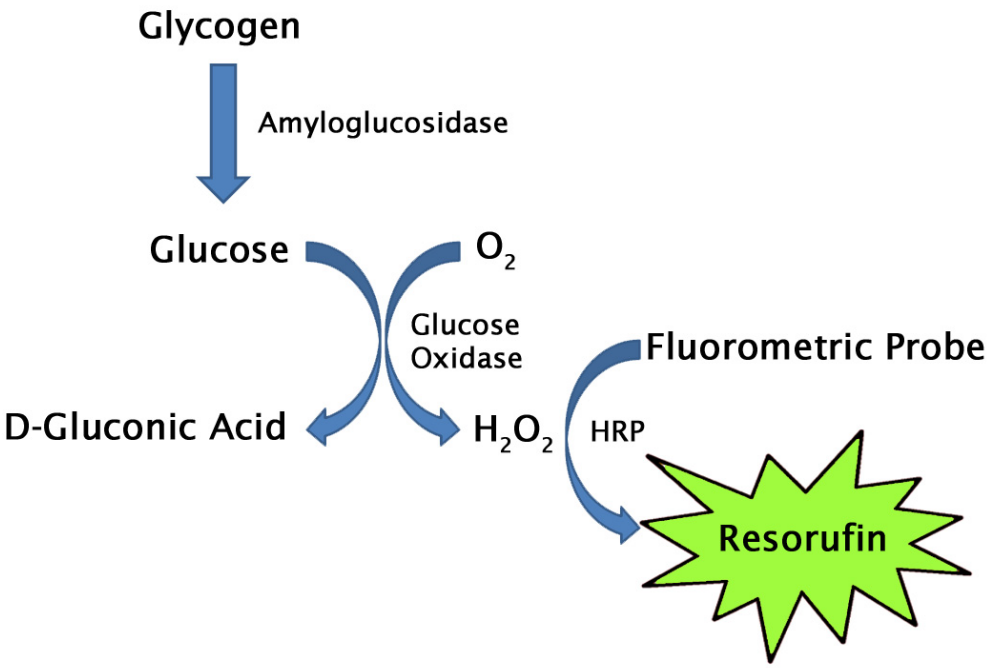

Figure 1. Glycogen Assay Principle.

Related Products

1. MET-5001: Lactose Assay Kit
2. MET-5013: Lactate Assay Kit (Fluorometric)
3. STA-399: Free Glycerol Assay Kit (Fluorometric)
4. STA-681: Glucose Assay Kit (Fluorometric)
5. STA-682: Total Carbohydrate Assay Kit
6. STA-674: Glutamate Assay Kit

Kit Components

1. Glycogen Standard (Part No. 50221C): One 50 µL tube at 3 mM.
2. 10X Assay Buffer (Part No. 268002): One 25 mL bottle of 500 mM sodium phosphate pH 7.4.
3. Fluorometric Probe (Part No. 50231C): One 50 µL tube in DMSO.
4. HRP (Part No. 234402-T): One 10 µL tube of a 100 U/mL solution in glycerol.
5. Amyloglucosidase (Part No. 50223C): One 1 mL tube at 15 U/mL.

   Note: One unit is defined as the amount of enzyme that will release 1.0 micromole of glucose per minute at pH 4.8 at 60°C.
6. Glucose Oxidase (Part No. 50015C): One 100 µL tube at 200 U/mL.

   Note: One unit is defined as the amount of enzyme that will oxidize 1.0 micromole of beta-D-glucose to D-gluconic acid and hydrogen peroxide per minute at pH 5.1 at 35°C.

Materials Not Supplied

1. Distilled or deionized water
2. Phosphate Buffered Saline (PBS)
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Standard 96-well fluorescence black microtiter plate and/or black cell culture microplate
6. Multichannel micropipette reservoir
7. Fluorescence microplate reader capable of reading excitation in the 530-570 nm range and emission in the 590-600 nm range.

Storage

Upon receipt, store the Glycogen Standard, Fluorometric Probe, HRP, Amyloglucosidase, and Glucose Oxidase at -20ºC. The Fluorometric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles. Store the 10X Assay Buffer at room temperature.

Note: After thawing Amyloglucosidase for the first time, make smaller aliquots and store at -20°C.

Preparation of Reagents

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity. Store at room temperature.
- Reaction Mix: Prepare a Reaction Mix by diluting the Fluorometric Probe 1:100, HRP 1:500, and Glucose Oxidase 1:50 in 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock solution, and 20 µL of Glucose Oxidase to 968 µL of 1X Assay Buffer for a total of 1 mL. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4ºC.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.

Preparation of Samples

- Cell culture supernatants: Cell culture media containing glucose should be avoided. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The cell conditioned media may be assayed directly or diluted as necessary in PBS.

  Note: Maintain pH between 7 and 8 for optimal working conditions as the Fluorometric Probe is unstable at high pH (>8.5).

- Tissue lysates: Sonicate or homogenize tissue sample in PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. The supernatant may be assayed directly or diluted as necessary in PBS.

- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in PBS.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed directly or diluted as necessary in PBS.

Notes:

- *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
- *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Fluorometric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).*
- *Avoid samples containing DTT or β-mercaptoethanol since the Fluorometric Probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh Glycogen standards before use by diluting in PBS. First, dilute the stock Glycogen Standard 3 mM solution 1:10 in PBS for a 300 µM Glycogen Solution. (e.g. add 5 µL of the stock 3 mM Glycogen Standard to 45 µL of PBS). Use the 300 µM Glycogen Solution to prepare a series of the remaining Glycogen standards according to Table 1 below.

Standard Tubes	300 µM Glycogen Solution (µL)	PBS (µL)	Glycogen (µM)	Glycogen (mg/dL)
1	25	975	7.5	0.5
2	250 of Tube #1	250	3.8	0.25
3	250 of Tube #2	250	1.9	0.13
4	250 of Tube #3	250	0.94	0.06
5	250 of Tube #4	250	0.47	0.03
6	250 of Tube #5	250	0.24	0.016
7	250 of Tube #6	250	0.12	0.008
8	0	250	0	0

Table 1. Preparation of Glycogen Standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each sample replicate requires two paired wells, one to be treated with Amyloglucosidase (+AG) and one without the enzyme (-AG) to measure endogenous glucose background (PBS will be added in place of Amyloglucosidase).
2. Add 50 µL of each glycogen standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 10 µL of Amyloglucosidase to the standards and to one half of the paired sample wells, and mix the well contents thoroughly.

4. Add 10 µL of PBS to the other half of the paired sample wells and mix thoroughly.
5. Incubate for 30 minutes at 37°C.
6. Add 50 µL of Reaction Mix to each well. Mix the well contents thoroughly and incubate for 45 minutes at 37ºC protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.

7. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.

Calculation of Results

1. Determine the average Relative Fluorescence Unit (RFU) values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without Amyloglucosidase (-AG) from the sample well values containing Amyloglucosidase (+AG) to obtain the difference. The fluorescence difference is due to the Amyloglucosidase activity.

$$\text{net RFU} = (\text{RFU}_{+\text{AG}}) - (\text{RFU}_{-\text{AG}})$$

5. Compare the net RFU of each sample to the standard curve to determine and extrapolate the quantity of glycogen present in the sample. Only use values within the range of the standard curve.

Example of Results

The following figures demonstrate typical Glycogen Assay Kit (Fluorometric) results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

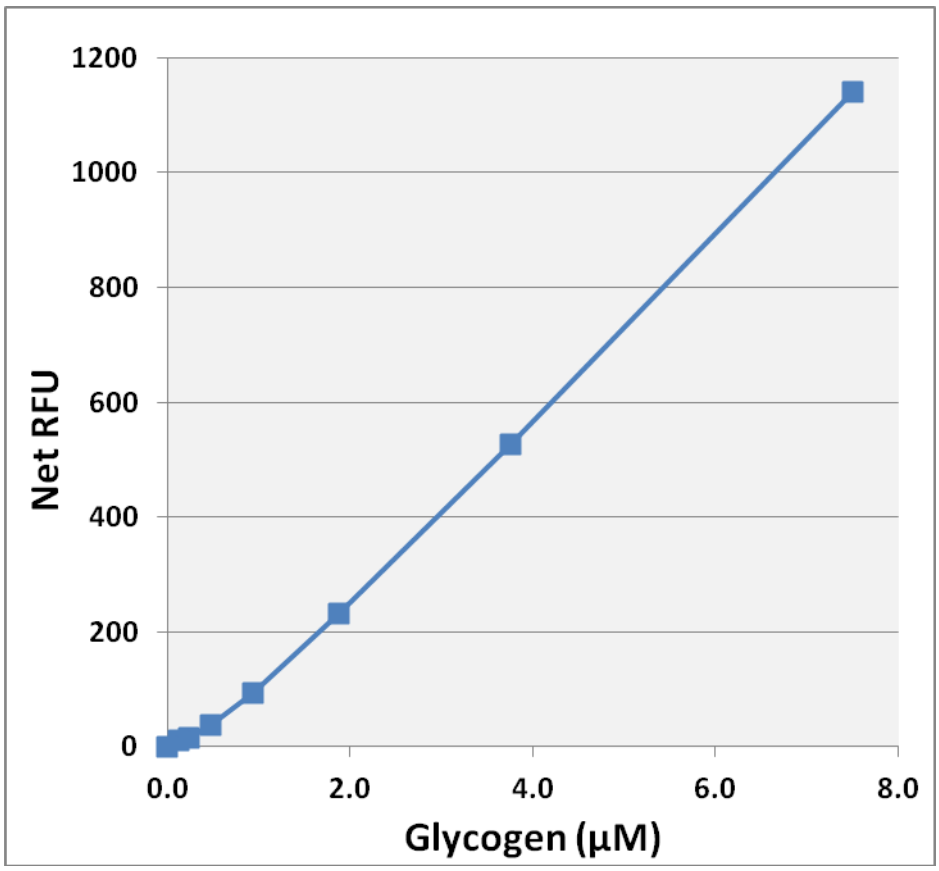

Figure 2: Glycogen Standard Curve.

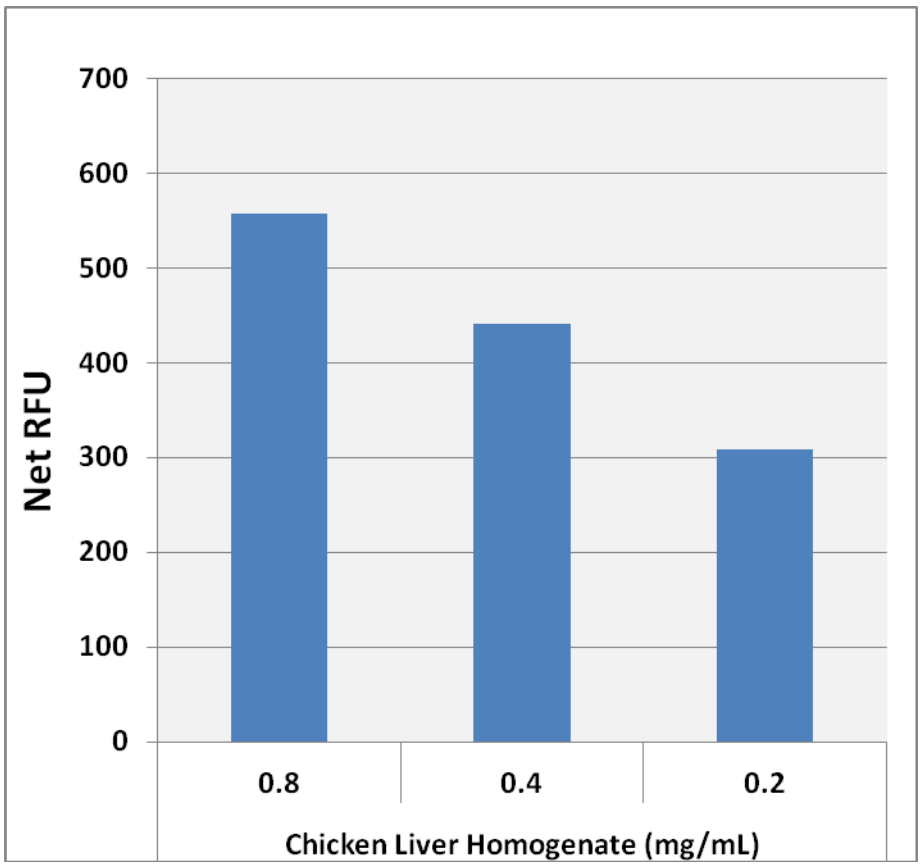

Figure 3: Glycogen Detection in Chicken Liver using the Glycogen Assay Kit (Fluorometric).

References

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3. Ferrer JC, et al. (2003) *FEBS Lett.* **546**:127.
4. Wolfsdorf JI, and Weinstein DA (2003) *Rev. Endocr. Metab. Disord.* **4**:95.
5. Bhattacharya K (2015) *Transl. Pediatr.* **20**:240-248.

Recent Product Citations

1. Noguchi, Y. et al. (2021). Microscopic image-based covariation network analysis for actin scaffold-modified insulin signaling. *iScience*. doi: 10.1016/j.isci.2021.102724.
2. Robb, J.L. et al. (2020). The metabolic response to inflammation in astrocytes is regulated by nuclear factor-kappa B signaling. *Glia*. doi: 10.1002/glia.23835.
3. Schweizer, S. et al. (2018). Substrate fluxes in brown adipocytes upon adrenergic stimulation and uncoupling protein 1 ablation. *Life Sci Alliance*. **1**(6):e201800136. doi: 10.26508/lsa.201800136.

Warranty

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