Glycogen Assay Kit

Glycogen Assay Kit is a detection kit for the quantification of Glycogen in tissue/cell lysate and other biological samples.

Catalog number: ARG82824

Package: 100 tests

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

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

Glycogen functions as one of two forms of energy reserves, glycogen being for short-term and the other form being triglyceride stores in adipose tissue (i.e., body fat) for long-term storage. In humans, glycogen is made and stored primarily in the cells of the liver and skeletal muscle. In the liver, glycogen can make up 5–6% of the organ's fresh weight, and the liver of an adult, weighing 1.5 kg, can store roughly 100–120 grams of glycogen. In skeletal muscle, glycogen is found in a low concentration (1–2% of the muscle mass) and the skeletal muscle of an adult weighing 70 kg stores roughly 400 grams of glycogen. The amount of glycogen stored in the body—particularly within the muscles and liver—mostly depends on physical training, basal metabolic rate, and eating habits (in particular oxidative type 1 fibres). Different levels of resting muscle glycogen are reached by changing the number of glycogen particles, rather than increasing the size of existing particles though most glycogen particles at rest are smaller than their theoretical maximum. Small amounts of glycogen are also found in other tissues and cells, including the kidneys, red blood cells, white blood cells, and glial cells in the brain. The uterus also stores glycogen during pregnancy to nourish the embryo. [Provide by Wikipedia: Glycogen]

PRINCIPLE OF THE ASSAY

This Glycogen Assay kit employs a simple colorimetric / fluorometric assay to measure the amount of glycogen in tissue / cell lysate and other biological samples. The assay uses a single Working Reagent that combines the enzymatic break down of glycogen and the detection of glucose in one step. Samples and standards are incubated and then read with a suitable microplate reader. The concentration of glycogen in the samples is then determined by comparing the absorbance value or Relative Fluorescence Units (RFU) of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, Store all components at -20°C upon receiving. Avoid multiple freeze / thaw cycles. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer	12 mL	-20°C
Standards (50 mg/mL)	50 µL	-20°C
Dye Reagent	120 µL	-20°C
Enzyme A (lyophilized)	1 vial	-20°C
Enzyme B	120 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microplate reader capable of reading excitation in the 530-nm range and emission in the 585 nm range
- Optical density plate reader
- 96-well plate or 96-well black plate
- Deionized or Distilled water
- Centrifuge tubes
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, Store all components at-20°C upon receiving. Avoid multiple freeze / thaw cycles.
- SH-group containing reagents (E.g., DTT, β-mercaptoethanol) may interfere with this assay and should be avoided in sample preparation.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Tissue Lysates: Sonicate or homogenize tissue sample in cold PBS and centrifuge at 14,000 x g for 5 minutes at 4°C. Use 10 µL clear supernatant for the assay.

Cell Lysates: Re-suspend cells at 1-2 x 10^6 cells/mL in PBS. Homogenize or sonicate the cells on ice. Centrifuge at 14,000 x g for 5 minutes at 4°C to remove debris. Use 10 µL clear supernatant for the assay.

Note:

1. Samples should be assayed immediately or stored at -80°C prior to performing the assay. Optimal experimental conditions for samples must be determined by the investigator.
2. SH-group containing reagents (E.g., DTT, β-mercaptoethanol) may interfere with this assay and should be avoided in sample preparation.

REAGENT PREPARATION

- **Enzyme A:** Add 120 µL of Assay Buffer to the Enzyme A tube. Make sure Enzyme A is fully dissolved by pipetting up and down. Store reconstituted Enzyme A at-20°C and use within 1 month.
- **Working Reagent:** For 10 assays, Mix 900 µL of Assay Buffer, 10 µL of Enzyme A, 10 µL of Enzyme B and 10 µL of Dye Reagent to a final volume of 930 µL. 90 µL for each reaction.
- **Blank Working Reagent:** For 10 assays, Mix 900 µL of Assay Buffer, 10 µL of Enzyme B and 10 µL of Dye Reagent to a final volume of 920 µL. 90 µL for each reaction.
- **Standards:** Prepare fresh Standards by first diluting the 50 mg/mL Standard stock solution 1:260 in deionized water. (E.g., Add 5 µL of Standard stock into 1245 µL of deionized water). Vortex thoroughly. This provides a 200 µg/mL working concentration. Use this working solution to prepare the Standards according to the Table below.

For colorimetric assay

Standard tubes	Final Glycogen conc. (µg/mL)	deionized water (µL)	Volume of 200 µg/mL working Standard (µL)
S1	200	0	200
S2	150	50	150
S3	100	100	100
S4	50	150	50
S0	0	200	0

For fluorometric assay

Standard tubes	Final Glycogen conc. (µg/mL)	deionized water (µL)	Volume of 200 µg/mL working Standard (µL)
S1	20	180	20
S2	15	185	15
S3	10	190	10
S4	5	195	5
S0	0	200	0

ASSAY PROCEDURE

Equilibrate all components to room temperature. During experiment, keep thawed enzymes in a refrigerator or on ice. Each Standard and sample should be assayed in duplicate or triplicate.

Colorimetric Procedure

1. Add **10 µL** of **samples** or serial **diluted Standards** into 96-well microplate.
   Note: If the sample contains glucose, transfer an additional 10 µL sample to another well as the Sample Blank.
2. Add **90 µL** of **Working Reagent** to each standard and sample well.
3. Add **90 µL** of **Blank Working Reagent** to each sample blank well.
4. Tap plate to mix. Incubate for **30 minutes** at **room temperature**.
5. Read the plate with an optical microplate reader at **O.D. 570 nm**.

Fluorometric Procedure

1. Follow step 1-4 of the colorimetric procedure. (Use the standard for fluorometric assay and 96-well black microplate instead)
2. Read the plate with a fluorescence microplate reader for excitation at 530 nm and for emission at 585 nm.

CALCULATION OF RESULTS

1. Linear detection range: 2 to 200 µg/mL glycogen for colorimetric assays and 0.2 to 20 µg/mL for fluorometric assays.
2. Calculate the average reading value (O.D. 570 nm or fluorescence intensity) for each set of standards and samples.
3. Subtract the Blank value from the standard reading values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the glycogen concentration of the sample.

$$\text{Glycogen} = (\text{Value}_{\text{sample}} - \text{Value}_{\text{Blank}}) / \text{Slope} \quad (\mu g/mL)$$

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Glycogen Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.