Glycogen Assay Kit

Catalog No.: abx294058

Size: 96 tests

Detection Range: 0.06 µg/ml – 4.0 µg/ml

Sensitivity: 0.06 µg/ml

Storage: Store all components at -20°C for up to 12 months. Store the Probe in the dark.

Application: For detection and quantification of Glycogen in animal liver and muscle tissue homogenates and cells.

Introduction

Glycogen is a polysaccharide made from glucose monomers, which join together using alpha-1-4 glycosidic bonds to form the linear chain, and alpha-1-6 glycosidic bonds to form the branch structures. Glycogen is a macromolecule which is used for energy storage by animals, as it can be broken back down into glucose when needed by the body, in a process known as glycogenolysis.

Abbexa's Glycogen Assay Kit is a quick, convenient, and sensitive method for measuring Glycogen content. Glycosidase enzymes catalyze the hydrolysis of glycosidic bonds in glycogen to release glucose molecules. The enzyme glucose oxidase catalyzes the oxidation of glucose, releasing hydrogen peroxide, which reacts with the kit components to produce a fluorescent substance. The fluorescence intensity at the excitation wavelength of 535 nm and emission wavelength of 587 nm is proportional to the Glycogen content.

Kit components

1. 96-well black microplate
2. Buffer A: 30 ml
3. Buffer B: 80 ml
4. Probe: 0.24 ml
5. Enzyme A: 1 vial
6. Enzyme B: 1 vial
7. Standard Solution (0.1 mg/ml): 0.5 ml
8. Plate sealer: 2

Materials required but not provided

1. Fluorescence microplate reader (Ex/Em=535 nm/587 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Ice
5. Pipettes and pipette tips
6. Incubator
7. Sonicator
8. Centrifuge and centrifuge tubes

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C, or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Carefully weigh at least 20 mg of tissue and wash in cold PBS (0.01 M, pH 7.4). Add 0.9 ml double-distilled water for each 0.1 g of tissue and homogenize manually using a mechanical homogenizer at 4°C in an ice water bath. Incubate at 95°C for 10 minutes. Centrifuge at 10,000 × g for 10 minutes, then carefully collect the supernatant and place on ice. Assay immediately, or store in liquid nitrogen.
- **Cell Samples:** Carefully prepare the number of cells needed for each assay (initial recommendation 10^6 cells). Wash cells with PBS (0.01 M, pH 7.4). Add 10^6 cells in 200 µl of double-distilled water to a new tube, then sonicate or mechanically homogenize. Incubate at 95°C for 10 minutes. Centrifuge at 12,000 × g for 10 minutes, then carefully collect the supernatant and place on ice. Assay immediately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Buffer A, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Rat liver tissue homogenate	3000-5000
10% Mouse muscle tissue homogenate	10-20

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Enzyme A Working Solution:**

  Add 1.2 ml of Buffer A into the Enzyme A vial to prepare Enzyme A Working Solution. Mix thoroughly to ensure that the Enzyme A is fully dissolved. Unused Enzyme A Working Solution can be stored at -20°C for up to 1 week.

- **Enzyme B Working Solution:**

  Add 240 µl of Buffer B into the Enzyme B vial to prepare Enzyme B Working Solution. Mix thoroughly to ensure that the Enzyme B is fully dissolved. Unused Enzyme B Working Solution can be stored at 2-8°C for up to 1 week.

- **Reaction Working Solution:**

  For each well, prepare 50 µl of Reaction Working Solution by thoroughly mixing 46 µl of Buffer B, 2 µl of Probe and 2 µl of Enzyme B Working Solution. The Reaction Working Solution should be prepared immediately before use. Avoid exposure to light.

- **25 µg/ml Standard Solution:**

  Dilute 100 µl of 0.1 mg/ml Standard Solution with 300 µl of Buffer A and mix thoroughly to prepare the 25 µg/ml Standard Solution.

- **Standards:** Label 7 tubes with 4.0 µg/ml, 3.0 µg/ml, 2.5 µg/ml, 2.0 µg/ml, 1.5 µg/ml, 1.0 µg/ml, and 0.5 µg/ml. Add 80 µl, 60 µl, 50 µl, 40 µl, 30 µl, 20 µl, and 10 µl of Standard (25 µg/ml) to the 4.0 µg/ml, 3.0 µg/ml, 2.5 µg/ml, 2.0 µg/ml, 1.5 µg/ml, 1.0 µg/ml, and 0.5 µg/ml tubes respectively, followed by 420 µl, 440 µl, 450 µl, 460 µl, 470 µl, 480 µl, and 490 µl of Buffer A, to prepare Standard Dilutions with concentrations 4.0 µg/ml, 3.0 µg/ml, 2.5 µg/ml, 2.0 µg/ml, 1.5 µg/ml, 1.0 µg/ml, and 0.5 µg/ml. These volumes are summarized in the following table:

Concentration (µg/ml)	**4.0**	**3.0**	**2.5**	**2.0**	**1.5**	**1.0**	**0.5**
25 µg/ml Standard (µl)	80	60	50	40	30	20	10
Buffer A (µl)	420	440	450	460	470	480	490

For the blank, or 0 µg/ml standard, use Buffer A. The volume of each standard will be 500 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

1. Mark standard, sample, and control wells on the microplate and record their positions. Each sample requires a corresponding control. *It is strongly recommended to prepare all the standards, samples and controls in duplicate.*
2. Add 50 µl of prepared standard concentrations to the standard wells.
3. Add 50 µl of sample to the sample wells.
4. Add 50 µl of control to the control wells.
5. Add 20 µl of Enzyme A Working Solution to the standard wells and sample wells.
6. Add 20 µl of Buffer A to the control wells.
7. Add 50 µl of Reaction Working Solution to all wells.
8. Tap the plate gently to mix thoroughly. Allow to stand at room temperature for 30 minutes in the dark.
9. Measure the fluorescence intensity at an excitation wavelength of 535 nm and an emission wavelength of 587 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Glycogen in each sample well can be derived with the following formulae:

1. Cell samples:

$$\text{Glycogen } (\mu g/10^6 \text{ cells}) = f \times \frac{V_1 \times (\Delta F - b)}{a \times n}$$

2. Tissue samples:

$$\text{Glycogen } (\mu g/\text{mg wet weight}) = f \times \frac{V_2 \times (\Delta F - b)}{a \times m}$$

where:

ΔF	Change in fluorescence intensity of sample, calculated as: (Sample Fluorescence Intensity - Control Fluorescence Intensity) - Blank Fluorescence Intensity
f	Dilution factor of sample before testing.
m	Mass of tissue sample (mg)
n	Number of cells ($\times 10^6$)
V_1	Volume of double-distilled water added during cell sample preparation (ml)
V_2	Volume of double-distilled water added during tissue sample preparation (ml)

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.