Glycogen Assay Kit (Colorimetric/Fluorometric)

LS-K183-100 (100 Tests) • **Store at -20°C**

Introduction

GLYCOGEN is a branched polysaccharide of glucose units linked by α- 1,4 glycosidic bonds and α-1,6 glycosidic bonds. It is stored primarily in the liver and muscle, and forms an energy reserve that can be quickly mobilized to meet a sudden need for glucose. The most common glycogen metabolism disorder is found in diabetes, in which, due to abnormal amounts of insulin, liver glycogen can be abnormally accumulated or depleted. Genetic glycogen storage diseases have been associated with various inborn errors of metabolism caused by deficiencies of enzymes necessary for glycogen synthesis or breakdown.

Simple, direct and automation-ready procedures for measuring glycogen concentrations find wide applications in research and drug discovery. LSBio's glycogen assay uses a single Working Reagent that combines the enzymatic break down of glycogen and the detection of glucose in one step. The color intensity of the reaction product at 570nm or fluorescence intensity at $\lambda_{ex/em}$ = 530/585 nm is directly proportional to the glycogen concentration in the sample. This simple convenient assay is carried out at room temperature and takes only 30 min.

Key Features

- Use as little as 10 µL samples. Linear detection range: 2 to 200 µg/mL glycogen for colorimetric assays and 0.2 to 20 µg/mL for fluorometric assays.

Components

Component	K183-100
	100 Tests
Assay Buffer	12 mL
Enzyme A	Dried
Enzyme B	120 µL
Dye Reagent	120 µL
Standard (50 mg/mL)	50 µL

Materials Not Supplied

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well Plates, optical density plate reader; black flat bottom uncoated 96-well plates, fluorescence plate reader.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

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LSBio
LifeSpan BioSciences, Inc.

Assay Procedure

Reagent Preparation

Reconstitute Enzyme A by adding 120 µL 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.

Sample Preparation

Samples can be prepared according to Murat & Serfaty (Clin Chem. 20:1576-1577, 1974). Briefly, homogenize tissue/cell sample in 25 mM citrate, pH 4.2, 2.5 g/L NaF on ice. Centrifuge 14,000 g for 5 min to remove debris, and use 10 µL clear supernatant for the assay.

Colorimetric Procedure

1. Equilibrate all components to room temperature. During experiment, keep thawed enzymes in a refrigerator or on ice.
2. Standards and samples: Dilute standard by mixing 5 µL Standard with 1.245 mL dH_2O to give 200 µg/mL standard. Dilute standard in dH_2O as follows.

No	200 µg/mL STD + H_2O	Vol (µL)	Glycogen (µg/ml)
1	200 µL + 0 µL	200	200
2	150 µL + 50 µL	200	150
3	100 µL + 100 µL	200	100
4	50 µL + 150 µL	200	50
5	0 µL + 200 µL	200	0

Transfer 10 µL standard and samples into separate wells of a clear flat-bottom microplate.

3. Working Reagent. For each reaction well, mix 90 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B and 1 µL Dye Reagent in a clean tube. Transfer 90 µL Working Reagent into each reaction well. Tap plate to mix.
4. Incubate 30 min at room temperature. Read optical density at 570 nm (550-585 nm).

Fluorometric Procedure

For fluorometric assays, the linear detection range is 0.2 to 20 µg/mL glycogen. Follow steps 1-3 of the colorimetric procedure, but prepare 0, 5, 10, 15 and 20 µg/mL Standard and use a black flat-bottom microplate. Incubate 30 min at room temperature and read fluorescence at λ_{ex} = 530 nm and λ_{em} = 585 nm.

Calculations

Subtract Blank reading (OD_{570nm} or fluorescence intensity) 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.

$$\text{Glycogen} = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope}} \ \mu g/mL$$

R_{SAMPLE} and R_{BLANK} are the OD_{570nm} or fluorescence intensity values of the sample and blank (water, or sample blank, see below).

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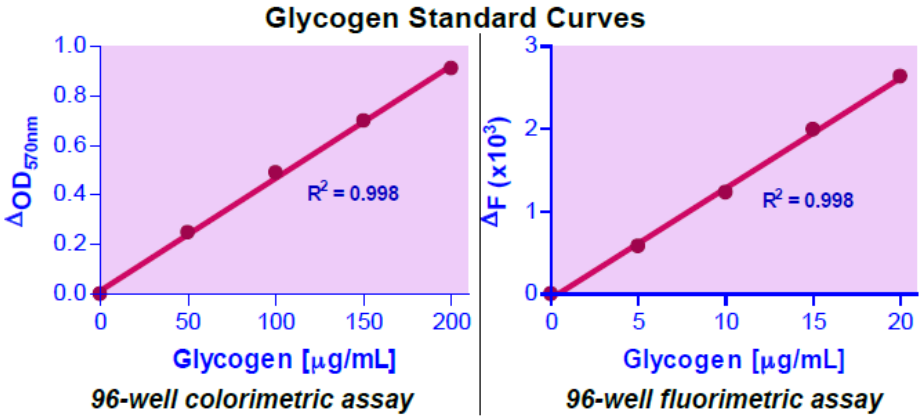
Sample Data



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