DATA SHEET

EnzyChrom™ Glycogen Assay Kit (Cat# E2GN-100)

Quantitative Colorimetric/Fluorimetric Glycogen Determination

DESCRIPTION

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. BioAssay Systems' 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 λem/ex = 585/530nm 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 fluorimetric assays.

KIT CONTENTS

Assay Buffer: 12 mL **Enzyme A:** 120 µL **Enzyme B:** 120 µL
Dye Reagent: 120 µL **Standard:** 50 µL 50 mg/mL

Storage conditions. The kit is shipped on ice. Store all reagents at -20°C. Shelf life of six months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

SAMPLE PREPARATION

Samples can be prepared according to established methods [1-3].

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 570nm (550-585nm).

FLUORIMETRIC PROCEDURE

For fluorimetric 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} = 530nm and λ_{em} = 585nm.

CALCULATION

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).

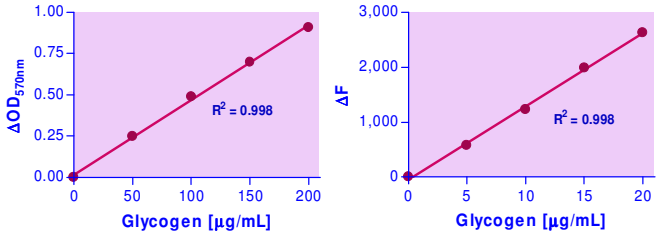
GENERAL CONSIDERATIONS

1. If the sample contains glucose, a Sample Blank well should be added: Prepare Sample Blank reagent by mixing 90 µL Assay Buffer, 1 µL Enzyme B, and 1 µL Dye Reagent (*No Enzyme A*). Add this reagent only to the Sample Blank wells. Subtract the OD or fluorescence of the Sample Blank from the sample readings to calculate glycogen concentration.
2. This assay is based on a kinetic reaction, the use of a multi-channel pipettor for adding the working reagent is recommended.
3. Interference. SH-group containing reagents (e.g., DTT, β-mercaptoethanol) may interfere with this assay and should be avoided in sample preparation.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices, centrifuge tubes, clear flat bottom 96-well plates and plate reader.

Glycogen Standard Curves



96-well colorimetric assay ***96-well fluorimetric assay***

LITERATURE

1. Murat JC, Serfaty A. (1974). Simple enzymatic determination of polysaccharide (glycogen) content of animal tissues. Clin Chem. 20(12):1576-1577.
2. Bueding, E. and Orrell, S.A. (1964). A Mild Procedure for the Isolation of Polydisperse Glycogen from Animal Tissues. J. Biol. Chem. 239: 4018-4020.
3. Dalrymple, R. H. and Hamm, R. (1973) A method for the extraction of glycogen and metabolites from a single muscle sample. Intl. J. Food Sci & Tech 8(4): 439-444.

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