

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

### Quantitative Colorimetric Glycogen Determination at 585nm

#### DESCRIPTION

GLYCOGEN is a branched polysaccharide of glucose units linked by  $\alpha$ -1,4 glycosidic bonds and  $\alpha$ -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  $\alpha$ -amylase/amyloglucosidase hydrolysis reagent that rapidly breaks down glycogen into glucose, which is determined with our proprietary colorimetric glucose assay reagent. This simple convenient assay is carried out at room temperature and takes 45 min.

#### KEY FEATURES

Use as little as 20  $\mu$ L samples. Linear detection range 0.9 to 50  $\mu$ g/mL (9 to 500 ng) glycogen.

#### KIT CONTENTS

**Assay Buffer:** 20 mL  
**Enzyme A:** 120  $\mu$ L  
**Enzyme B:** 220  $\mu$ L  
**Standard:** 50  $\mu$ L 50 mg/mL Standard  
**Detection Reagent:** 20 mL

**Storage conditions.** The kit is shipped on dry ice. Store Standard and Detection Reagent at 4°C and other reagents at -20°C. Shelf life of three 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.

#### PROCEDURES

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

**Reagent Preparation:** Bring all reagents to room temperature. Keep thawed enzyme tubes on ice or in refrigerator during the experiment.

#### Assay Procedure

1. Dilute standard by mixing 5  $\mu$ L Standard with 5 mL H<sub>2</sub>O to give 50  $\mu$ g/mL standard. Transfer 10  $\mu$ L H<sub>2</sub>O and 10  $\mu$ L diluted standard into wells of a clear flat-bottom 96-well plate.

For each sample, transfer 10  $\mu$ L sample into two separate wells, one well serves as a sample blank control for background glucose assay and the other well for total glucose assay.

2. Prepare appropriate working reagents as follows,  
*For H<sub>2</sub>O Blank, Standard and Sample wells:* prepare hydrolysis reagent by mixing 42  $\mu$ L Assay Buffer, 1  $\mu$ L Enzyme A and 1  $\mu$ L Enzyme B.

*For sample blank wells:* prepare control reagent by mixing 43  $\mu$ L Assay Buffer and 1  $\mu$ L Enzyme B (No Enzyme A). Add this reagent only to the Sample Control wells.

Add 40  $\mu$ L appropriate reagent per well. Tap plate to mix and incubate for 15 min at room temperature.

3. Add 150  $\mu$ L Detection Reagent to all wells. Incubate 30 min and measure optical density at 585nm (540-610nm).

*Note:* if Sample OD values are higher than that of the standard, dilute sample in Assay Buffer, repeat assay and multiply results by the dilution factor.

#### CALCULATION

The glycogen concentration of Sample is calculated as

$$[\text{Glycogen}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{OD}_{\text{STANDARD}} - \text{OD}_{\text{H}_2\text{O}}} \times 50 \text{ } (\mu\text{g/mL})$$

OD<sub>SAMPLE</sub>, OD<sub>BLANK</sub>, OD<sub>STANDARD</sub> and OD<sub>H<sub>2</sub>O</sub> are optical density values of the sample, Sample Blank, the 50  $\mu$ g/mL Standard and H<sub>2</sub>O.

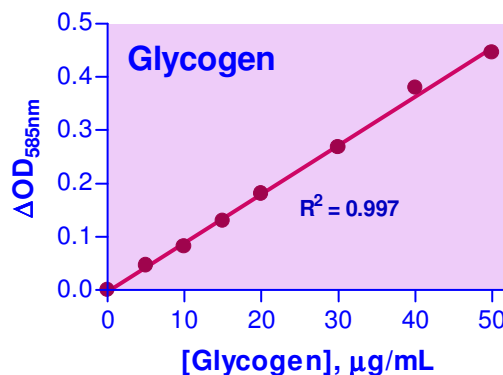
#### GENERAL CONSIDERATIONS

1. This assay is based on a kinetic reaction, the use of a multi-channel pipettor for adding the hydrolysis and detection reagent is recommended.

2. Interference. Several chemicals are known to interfere and should be avoided in sample preparation. These include ascorbic acid, EDTA, heparin, NP-40 (>0.6%), SDS (>0.12%) and Tris (>8 mM).

#### MATERIALS REQUIRED, BUT NOT PROVIDED

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



Standard Curve in 96-well plate assay

#### LITERATURE

- Murat JC, Serfaty A. (1974). Simple enzymatic determination of polysaccharide (glycogen) content of animal tissues. Clin Chem. 20(12):1576-1577.
- 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.
- 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.