

## **Technical Manual**

## **ColorFluor Glycogen Assay Kit**

**Catalogue Code: BA0065**

**Pack Size: 100 assays**

**Research Use Only**

## 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. The Assay Genie ColorFluor Glycogen Assay Kit 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  $\mu$ L samples. Linear detection range: 2 to 200  $\mu$ g/mL glycogen for colorimetric assays and 0.2 to 20  $\mu$ g/mL for fluorimetric assays.

## KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

<b>Assay Buffer:</b>	12 mL	<b>Dye Reagent:</b>	120 $\mu$ L
<b>Enzyme A:</b>	Dried	<b>Standard:</b>	50 $\mu$ L 50 mg/mL
<b>Enzyme B:</b>	120 $\mu$ L		

**Storage conditions.** The kit is shipped on ice. Store all components at  $-20^{\circ}\text{C}$  upon receiving. Shelf life: 6 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

### Reagent Preparation:

Reconstitute Enzyme A by adding 120  $\mu$ L Assay Buffer to the Enzyme A tube. Make sure Enzyme A is fully dissolved by pipetting up and down. Store reconstituted Enzyme A at  $-20^{\circ}\text{C}$  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  $\mu$ L 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  $\mu$ L Standard with 1.245 mL dH<sub>2</sub>O to give 200  $\mu$ g/mL standard. Dilute standard in dH<sub>2</sub>O as follows.

No	200 $\mu$ g/mL STD + H <sub>2</sub> O	Vol ( $\mu$ L)	Glycogen ( $\mu$ g/ml)
1	200 $\mu$ L + 0 $\mu$ L	200	200
2	150 $\mu$ L + 50 $\mu$ L	200	150
3	100 $\mu$ L + 100 $\mu$ L	200	100
4	50 $\mu$ L + 150 $\mu$ L	200	50
5	0 $\mu$ L + 200 $\mu$ L	200	0

Transfer 10  $\mu$ L standard and samples into separate wells of a clear flat-bottom microplate.

3. *Working Reagent.* For each reaction well, mix 90  $\mu\text{L}$  Assay Buffer, 1  $\mu\text{L}$  Enzyme A, 1  $\mu\text{L}$  Enzyme B and 1  $\mu\text{L}$  Dye Reagent in a clean tube. Transfer 90  $\mu\text{L}$  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).

**Fluorimetric Procedure:**

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

**CALCULATION**

Subtract Blank reading (OD570nm or fluorescence intensity) from the standard reading values and plot the  $\Delta\text{OD}$  or  $\Delta\text{F}$  against standard concentrations. Determine the slope and calculate the glycogen concentration of the sample.

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

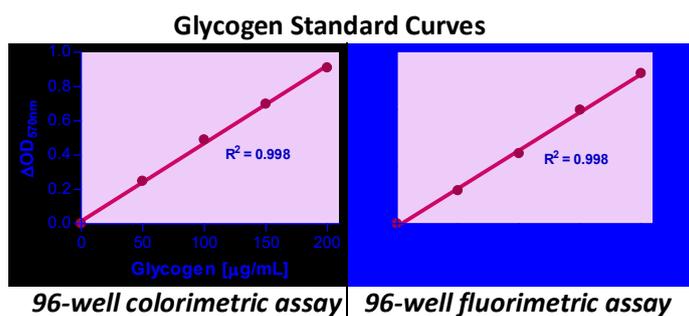
$R_{\text{SAMPLE}}$  and  $R_{\text{BLANK}}$  are the OD570nm or fluorescence intensity values of the sample and blank (water, or sample blank, see below).

**GENERAL CONSIDERATIONS**

1. If the sample contains glucose, a Sample Blank well should be added: Prepare Sample Blank reagent by mixing 90  $\mu\text{L}$  Assay Buffer, 1  $\mu\text{L}$  Enzyme B, and 1  $\mu\text{L}$  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. Interference. SH-group containing reagents (e.g., DTT,  $\beta$ -mercaptoethanol) may interfere with this assay and should be avoided in sample preparation.

**MATERIALS REQUIRED, BUT NOT PROVIDED**

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates (e.g. VWR cat# 82050-760), optical density plate reader; black flat-bottom uncoated 96-well plates (e.g. VWR cat# 82050-676), fluorescence plate reader.



**PUBLICATIONS**

1. Iwabuchi S, and Kawahara K (2011). Inducible astrocytic glucose transporter-3 contributes to the enhanced storage of intracellular glycogen during reperfusion after ischemia. *Neurochem Int* 59(2):319-325.
2. Primassin, S., et al (2011). Hepatic and muscular effects of different dietary fat content in VLCAD deficient mice. *Mol Genet Metab* 104(4): 546-551.
3. Yin, M et al (2011). Metformin improves cardiac function in a nondiabetic rat model of post-MI heart failure. *Am J Physiol Heart Circ Physiol* 301(2):H459-468.

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