USER GUIDE

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Version 2.0

糖原检测试剂盒

Glycogen Assay Kit

Do not eat Store at -20° C & in the dark.

Cat.No.MKG3985 **Size :** 100 tests

Technical literature is available at : www.mesgenbio.com
E-mail MesGen Technical Services if you have questions on use of this system : tech@mesgenbio.com

Application

For quantitative determination of glycogen and evaluation of drug effects on glycogen metabolism.

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. MesGen Biotechnology ' glycogen assay uses a single Working Reagent that combines the enzymatic break down of glycogen and the detection of glucose. The color intensity of the reaction product at 570nm or fluorescence intensity at lex/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

Sensitive and accurate. Use as little as 10 µL samples. Linear detection range in 96-well plate: 4 to 2000 µg/mL glycogen for colorimetric assays and 0.4 to 200 µg/mL for fluorimetric assays. Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 5-10 min at room temperature.

Samples

Serum, plasma, urine, saliva, milk, culture medium, food, agriculture etc

Kit contents

Reagent A : 5 mL **Reagent B : 5** mL
Reagent C : 2 mL **Reagent D : 200** µL
20mg/mL **Glycogen Standard:** 1 mL

Procedures

Sample Preparation:

Samples can be prepared according as follow.

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 reagents in a refrigerator or on ice.
2. Standards and samples: Dilute standard by mixing 30 µL Standard with 270 µL dH_2O to give 2000 µg/mL standard. Dilute standard in dH_2O as follows.

NO.	2000 µg/mL STD + H_2O	Vol (µL)	Glycogen (µg/ml)
1	100 µL + 0 µL	100	2000
2	60 µL + 40 µL	100	1200
3	40 µL + 60 µL	100	800
4	20 µL + 80 µL	100	400
5	10 µL + 90 µL	100	200
6	5 µL + 95 µL	100	100
7	1 µL + 99 µL	100	20
8	0 µL + 100 µL	100	0

Notice : Transfer 10 µL standard and samples into separate wells of a clear flat-bottom microplate. If the sample contains glucose, transfer an additional 10 µL sample to another well for the Sample Blank.

3. Transfer 20 µL Reagent C to each Sample, dH_2O Blank and Standard well. Tap plate to mix. If the sample contains glucose, transfer 20 µL dH_2O to the Sample Blank well instead of Reagent C. Incubate 5 min at room temperature.
4. Working Reagent. For each reaction well, mix 50 µL Reagent

A, 50 µL Reagent B, and 2 µL Reagent D. Transfer 100 µL Working Reagent into each Standard and Sample well (including Sample Blank well).Tap plate to mix.

4. Incubate 5-10 min at room temperature. Read optical density at 570 nm (550-585 nm).

Fluorimetric Procedure:

For fluorimetric assays, the linear detection range is 0.4 to 200 µg/mL glycogen. Follow steps of the colorimetric procedure, but prepare 0, 5, 10, 15 and 20 µg/mL Standard and use a black flat-bottom microplate.Incubate 5-10 min at room temperature and read fluorescence at lex = 530nm and lem = 585 nm.

Calculation

Subtract Blank reading (OD570nm or fluorescence intensity) from the standard reading values and plot the DOD or DF 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 OD570nm or fluorescence intensity values of the sample and blank (water, or sample blank).

GENERAL CONSIDERATIONS

1. This assay is based on a kinetic reaction, the use of a multi-channel pipettor for adding the working reagent is recommended.
2. SH-group containing reagents (e.g., DTT, β-mercaptoethanol) may interfere with this assay and should be avoided in sample preparation.

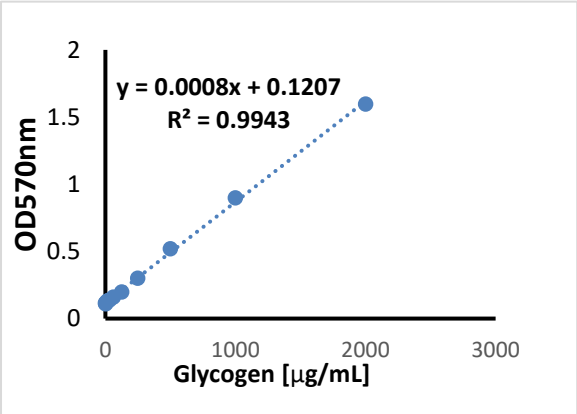
Storage conditions

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

Shelf life

6 months after receipt.

Glycogen Standard Curves for Reference



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