MANUAL

Glycogen Assay Kit (Colorimetric)

Catalog #: MA-GLYC

ISO 13485:2016

Introduction

Glycogen is a polysaccharide found in animals as well as simpler organisms such as fungi. Glycogen is made up of glucose monomers and is the primary method of storing glucose in animals. It is stored primarily in the liver and muscle, forming an energy reserve that can be quickly mobilized to meet sudden needs for glucose. The most common glycogen metabolism disorder is found in diabetes, where abnormal insulin levels cause liver glycogen to be abnormally accumulated or depleted. Genetic glycogen storage diseases are associated with various inborn errors of metabolism caused by deficiencies of enzymes necessary for glycogen synthesis or breakdown.

Glycogen Assay Kit provides a simple, reproducible, and sensitive tool of measuring glycogen concentration in cell lysates, urine, saliva, and other biological liquid samples. This assay employs a coupled enzymatic reaction system that combines multiple steps. Initially, glycogen is hydrolyzed by amyloglucosidase to form β-D-glucose, which is then specifically oxidized by glucose oxidase to form hydrogen peroxide (H_2O_2). Peroxidase then catalyzes the reaction of hydrogen peroxide with the Colorimetric Probe to form a pink colored product. The optical density measured at 510nm is directly proportional to the concentration of glycogen present in the sample.

Storage

The entire kit should be stored below -20 °C for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate	A 96-well (12 strips x 8 wells) plate	RT*
Assay Buffer	25 ml	2–8 °C, 3 months
Hydrolysis Buffer	5 ml	2–8 °C, 3 months
Glycogen Standard	2 vials (Lyophilized)	Do not store and reuse.
Amyloglucosidase	2 vials (Lyophilized)	Do not store and reuse.
Enzyme Mix	2 amber vials (Lyophilized)	Do not store and reuse.
Colorimetric Probe	2 amber vials (Lyophilized)	Do not store and reuse.

RT = room temperature

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 510 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Tubes to prepare sample dilutions
4. Incubator at 37°C
5. 50 ml conical tubes

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Urine, Saliva samples:

To remove insoluble particles, spin at 10,000 x g for 5 min. The supernatant may be assayed directly.

NOTE:
Optimal experimental conditions for samples must be determined by the investigator. A set of serial dilutions is recommended for samples to achieve optimal assay results.

If the calculated glycogen concentration of the sample is higher than 200 µg/mL, dilute the sample in Assay Buffer and repeat the assay.

Reagent Preparation

REAGENT	PREPARATION
Microplate	No preparation
Assay Buffer	
Hydrolysis Buffer	
Glycogen Standard	See “Standard Preparation” section
Amyloglucosidase	For each tube, spin down briefly, add 150 µL Hydrolysis Buffer, mix well. Then transfer everything into a new Eppendorf tube filled with 500 µL Hydrolysis Buffer, the final volume is 650 µL. This is **Amyloglucosidase Solution**. This is enough to assay 60 wells. Prepare the additional vial as needed.
Enzyme Mix	For each tube, spin down briefly, add 1 mL Assay Buffer, mix well.
Colorimetric Probe	For each tube, spin down briefly, add 1 mL Assay Buffer, mix well.
Glucose Reagent	Add 1 mL reconstituted Enzyme Mix, and 1 mL reconstituted Colorimetric Probe into 6 mL Assay Buffer to make the **Glucose Reagent**. This is enough to assay 55 wells. Prepare the additional vial as needed. The **Glucose Reagent** is stable for 1 day at 2-8°C in the dark.

Standard Preparation

To prepare a dilution series of Glycogen Standard in the concentration range of 0 µg/mL – 200 µg/mL (see Table below),

1. Label 8 microtubes #1 through 8 with the following concentrations: 200, 100, 50, 25, 12.5, 6.25, 3.125, 0 µg/mL.
2. Pipette 225 µL Assay Buffer into labeled tube #1, and 100 µL Assay Buffer into labeled tube #2 through #8.
3. Briefly spin down glycogen standard tube provided, add 50 µL assay buffer to make 2 mg/mL stock, mix well.
4. Pipette 25 µL of 2 mg/mL stock into tube#1, mix thoroughly and quick spin, to make a 200 µg/mL standard.
5. To make the 100 µg/mL standard, pipette 100 µL of tube #1 into the tube labeled #2. Mix thoroughly and quick spin.
6. Repeat this step with each successive concentration, preparing a dilution series as shown in the Table below. Each time, use 100 µl of the prior concentration until the 3.125 µg/mL is reached. Mix each tube thoroughly before the next transfer. Standards should be prepared fresh and used immediately.

Labeled Tubes	Glycogen Standard (µL)	Assay Buffer (µL)	Standard Conc. (µg/mL)
1	25 µL of 2mg/ml stock	225 µL	200
2	100 µL of Tube #1	100 µL	100
3	100 µL of Tube #2	100 µL	50
4	100 µL of Tube #3	100 µL	25
5	100 µL of Tube #4	100 µL	12.5
6	100 µL of Tube #5	100 µL	6.25
7	100 µL of Tube #6	100 µL	3.125
8	0µL	10 µL	0

Assay Procedure

Plate Set Up

A representative plate layout is given below. Measuring all samples in duplicate is recommended.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1	S5	S5	S9	S9	S13	S13	S17	S17
B	B	B	B1	B1	B5	B5	B9	B9	B13	B13	B17	B17
C	C	C	S2	S2	S6	S6	S10	S10	S14	S14	S18	S18
D	D	D	B2	B2	B6	B6	B10	B10	B14	B14	B18	B18
E	E	E	S3	S3	S7	S7	S11	S11	S15	S15	S19	S19
F	F	F	B3	B3	B7	B7	B11	B11	B15	B15	B19	B19
G	G	G	S4	S4	S8	S8	S12	S12	S16	S16	S20	S20
H	H	H	B4	B4	B8	B8	B12	B12	B16	B16	B20	B20

A-H = Standards S1-S20 = Sample 1-20 B1-20 = Sample Background 1-20

Assay Protocol

Each Glycogen Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 20 µL per well of the diluted Glycogen Standards or samples to the 96-well microtiter plate.

   NOTE: Each sample replicate requires two paired wells per replicate, one to be treated with Amyloglucosidase (Sample Wells) and one without the enzyme to measure endogenous glucose background (Sample Background Wells). For example, if samples will be assayed in duplicate, each sample will be added to four wells total.

2. For Standard Wells and Sample Wells: add 10 µL of the Amyloglucosidase Solution (See "Reagent Preparation") to the corresponding wells.
   For Sample Background Wells: add 10 µL of hydrolysis buffer to corresponding wells.
   Carefully shake the plate for a few seconds to mix. Incubate the plate at 37ºC for 30 minutes with gentle mixing in the dark.
3. Add 150ul of Glucose Reagent (See "Reagent Preparation") to each well. Incubate the plate at 37ºC for 30 minutes with gentle mixing in the dark.
4. Measure the absorbance at 510nm using a plate reader.

Calculation of Results

Subtract the blanks and Sample Background

1. Calculate the average absorbance values for each set of standards and samples. Subtract the average absorbance of the blank wells (Standard 0 µg/mL) from the absorbance value of all other wells. These are the corrected absorbance.
2. For each sample: subtract the corrected absorbance of Sample Background from the corrected absorbance of Sample to get the corrected sample measurement (CSM).
   $OD_{CSM} = OD_{Sample} - OD_{Background}$

Plotting the standard curve

Make a plot of the corrected absorbance of the standards at 510nm as a function of glycogen concentration.

Determination of sample glycogen concentration

Glycogen (µg/mL) = $\frac{OD_{CSM} - (y\_intercept)}{Slope} \times DF$

OD_{CSM} = Optical density (OD) reading of the Corrected Sample Measurement

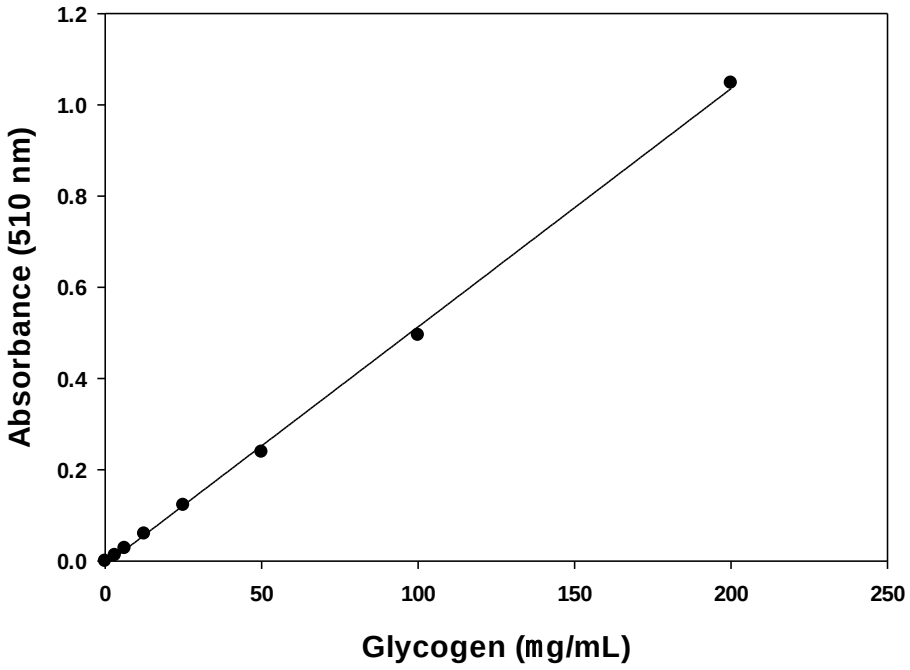
y_intercept = Intercept of y axis of the linear regression of the standard curve

Slope is from the plot of glycogen concentration vs. Absorbance (as shown in Typical data below).

DF = Sample Dilution factor (DF = 1 for undiluted Samples)

Note: If the calculated glycogen concentration of the sample is higher than 200 µg/mL, dilute the sample in assay buffer and repeat the assay.

A. Typical Data



These standard curves are for demonstration only. A standard curve must be run with each assay.

B. Sensitivity

The minimum detectable concentration of glycogen is 2.1 µg/mL.
Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank.

C. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 1.1%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 2.5%.

This product is for research use only.