MA-0112

Glycogen Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Background Information:

Glycogen, the main form of glucose storage in animals is synthesized and stored primarily in liver and muscle. Glycogen is formed on a core of glycogenin which links the first few glucose molecules after which other enzymes add glucose monomers to the growing polymer with α-1,4 linkages extending linear chains, and α-1,6 linkages giving a branched structure. A number of diseases are related to the inability to store or utilize glycogen properly. AkrivisBio's Glycogen Assay is a simple sensitive method to measure glycogen levels in biological samples with a sensitivity from the low nanogram to high microgram level.

Assay Principle:

1 – Glycogen is hydrolyzed in the presence of glucoamylase, also known as maltase or alpha glucosidase to glucose monomers
2 – Glucose is oxidized by glucose oxidase forming hydrogen peroxide
3 – Hydrogen peroxide is utilized by peroxidase to oxidize ADHP to resorufin, resulting in intense color and fluorescence.

Assay Components:

Hydrolysis Buffer	25 ml	NM	MA-0112A
Assay Buffer	25 ml	WM	MA-0112B
ADHP Solution	0.2 ml	Red	MA-0112C
Amyloglucosidase	lyoph	Blue	MA-0112D
Glucose Oxidase/HRP	lyoph	Green	MA-0112E
Glycogen Standard	100 µl	Yellow	MA-0112F

User Supplied Materials:

Plate shaker
Homogenizer or Polytron

Storage and Handling: Store the kit at -20°C protected from light. Briefly centrifuge small vials prior to opening.
Hydrolysis Buffer: Warm to room temperature before use. Store at 4°C.
Assay Buffer: Warm to room temperature before use. Store at or 4°C.
ADHP Solution: Ready to use as supplied. DMSO freezes just below room temperature. Bring to room temperature before use. Store at –20°C.
Amyloglucosidase: Reconstitute with 220 µl of Hydrolysis Buffer and mix gently to dissolve. Keep on ice. Store at –20°C.
Glucose Oxidase/HRP: Reconstitute with 220 µl of Assay Buffer and mix gently to dissolve. Keep on ice. Store at –20°C.
Glycogen Standard: 2 mg/ml. Store at -20°C

Glycogen Assay Protocol:

1. Standard Curve:

Absorbance-based assay: Dilute the standard by adding 8 µl to 192 µl of DI water to give 80 µg/ml. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl of the Standard to wells of a 96-well plate. Adjust all wells to 50 µl with Hydrolysis Buffer, giving 0 - 0.4 - 0.8 - 1.2 - 1.6 - 2.0 µg glycogen per well.

Fluorescence-based assay: Dilute the Standard as for the absorbance-based assay, then 10X more (to 8 µg/ml) by adding 10 µl of the Standard to 90 µl of DI water. Add 0 – 5 – 10 – 15 – 20 - 25 µl to wells of a 96-well plate. Adjust all wells to 50 µl with Hydrolysis Buffer to generate 0 – 40 – 80 – 120 - 160 - 200 ng glycogen per well.

2. Sample Preparation: Liquid samples can be used directly. For cells (10^6), or tissue (10 mg) homogenize quickly with 200 µl H_2O. Boil the homogenates 5-10 min. to inactivate enzymes. Centrifuge at 16,000 x g for a few minutes to remove insoluble material. Use the supernatant in the assay. Add 2-50 µl samples to pairs of wells of a 96-well plate. One will serve as a glucose background control. Adjust all well volumes to 50 µl with Hydrolysis Buffer.

Note: Glycogen is metabolized very rapidly (within seconds) in some tissues after death so special care must be taken to minimize glycogen loss when taking tissue samples, such as freezing samples immediately, keeping cold and working efficiently. There are several methods for glycogen recovery from tissues depending upon the tissue or information desired. We recommend consulting the literature to determine the best method for your purposes. See the note at the end of this protocol.

3. Hydrolysis: Add 2 µl Amyloglucosidase to all Standard and sample wells. **Do not add Amyloglucosidase** background controls. Incubate 30 min. at RT.

4. Oxidation Reaction Mix: Prepare 50 µl of Mix for each sample and Standard. When pipetting to a partial plate it is useful to have a slight excess so make enough for standards and samples plus one more well. For each well prepare the following:

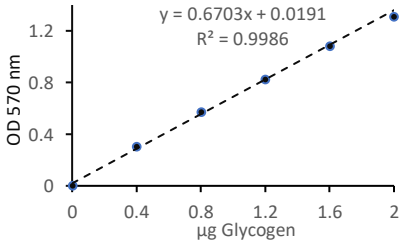
	Absorbance Based Assay	Fluorescence Based Assay
Assay Buffer	46 µl	48.7 µl
Glucose Oxidase/HRP	2 µl	1.0 µl
ADHP Solution	2 µl	0.3 µl

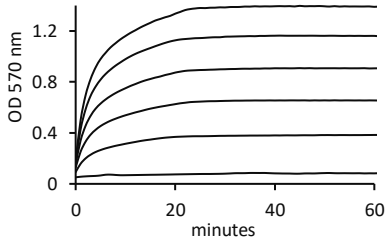
Add 50 µl of the Oxidation Reaction Mix to each well sample, background control and standard well.

5. Measurement: Monitor the reaction progress with either absorbance (OD 570 nm) or fluorescence (Excitation/Emission = 535/587 nm). When the signal from the standards is no longer changing, the reaction is finished. It should take less than 30 minutes to reach the endpoint.

6. Typical Results

µg Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.0582	0.0526	0	0
0.4	0.2555	0.2469	0.1973	0.1943
0.8	0.4698	0.4648	0.4116	0.4122
1.2	0.6763	0.6815	0.6181	0.6289
1.6	0.9259	0.9432	0.8677	0.8906
2.0	1.1458	1.1225	1.0876	1.0699





7. Calculation: Correct background by subtracting the 0 glycogen standard from all other standard readings. Plot Glycogen Standard Curve. Determine the slope of the standard curve which defines the OD/µg. Subtract glucose background values from each paired sample. This absorbance (fluorescence) is attributable to glucose derived from glycogen. Apply standard curve slope to background corrected sample values to convert from OD to to µg glycogen in the well. To get glycogen per sample:

A. Glycogen in well/sample volume added to well = glycogen/µl sample
B. Glycogen/µl sample X total sample supernatant volume after homogenization and centrifugation = total glycogen extracted from sample
C. Total glycogen extracted from sample / mg tissue (or number of cells) = glycogen/mg sample (/# of cells).

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MA-0112 Glycogen Assay: Notes on Glycogen Isolation

Sample Preparation:

There are a variety of methods for extraction of glycogen from tissues depending upon a) the type of tissue the glycogen is to be extracted from and b) the type of information desired. The gentlest procedure is the method referred to in reference 1, which maintains the molecular weight of the glycogen so that analysis of the molecular distribution is possible.

A rapid method useful for small tissue samples is detailed in reference 4. Basically, a small sample of tissue is homogenized in 50 volumes of distilled water, diluted appropriately and immediately used in the assay. Since endogenous glucose will be a significant factor utilizing this method, a glucose background control must be conducted where the sample is directly placed in development buffer with development enzyme mix (without prior treatment with the hydrolysis reagents). If the sample will not be immediately assayed, it should be placed in a capped, vented microcentrifuge tube and boiled for 5 min to inactivate any enzyme activities present and stored at -20°C until assayed. Samples from high content tissues (liver, muscle) prepared in this way should have sufficient glycogen such that 5-25 µl aliquots will give a clearly measurable colorimetric signal. If the sample is from low content tissues, either take a large aliquot (50µl) for the colorimetric assay or a proportionally smaller aliquot (10-25µl) in the fluorometric assay.

Caveats:

1) in some tissues such as neural tissue, very rapid rates of anaerobic metabolism continue after death causing rapid declines in glucose to undetectable levels within a few seconds. Utilization of glycogen follows and large decreases in glycogen content are seen within less than a minute. Thus, accurate measurement of glycogen in such tissues requires very rapid quenching of metabolic activity such as freeze clamp or immediate removal of tissue to liquid nitrogen followed by grinding in the liquid nitrogen and storage at -20 or -80°C until used.

2) In some samples i.e., Saccharomyces, glycogen is distributed between soluble and insoluble pools. It is not clear that both pools are completely hydrolyzed.

If the sample to be analyzed is sufficiently large (a few hundred milligrams to grams of tissue), a more quantitative method is as follows:

Take tissue or cells to a final content of 30-50% in 30% KOH. Heat to 100°C for 2 hours, cool and add 2 volumes of 95% ethanol. This will precipitate the crude glycogen. Centrifuge and collect the precipitate. Dissolve/suspend the precipitate in a minimal amount of distilled water and acidify to pH 3 with HCL (5N). Reprecipitate with 1 volume of ethanol. Repeat wash/acidification/precipitation 2 more times, then wash precipitate with ethanol and dry. This procedure removes the vast majority of the glucose background with minimal effect on the glycogen. The dried material can be weighed and dissolved/suspended in hydrolysis buffer for analysis.

I. References:

1) E.Bueding and S.A. Orrell (1964) A Mild Procedure for the Isolation of Polydisperse Glycogen from Animal Tissues. J. Biol. Chem. 239, 12, pp 4018-4020

2) R. H. Dalrymple, R. Hamm (1973) A method for the extraction of glycogen and metabolites from a single muscle sample. Intl J of Food Sci & Tech, 8, 4 pp 439-444

3) G. Cappeln, F. Jessen (2002) ATP, IMP, and Glycogen in Cod Muscle at Onset and During Development of Rigor Mortis Depend on the Sampling Location. J. Food Sci. 67, #3, pp 991-995

4) Huijing, F. (1970) A Rapid Enzymic Method For Glycogen Estimation In Very Small Tissue Samples., Clin. Chim. Acta. 30, pp 567-572.

5) Monique Rousset, etc. (1981) Presence of Glycogen and Growth-related Variations in 58 Cultured Human Tumor Cell Lines. Cancer Research. 41, 1165-1170.