MA-0136

Glycogen Assay II

(100 wells, Colorimetric, OD 450 nm, Store at -20°C)

Introduction:

Glycogen is the primary storage form of glucose. The facile interconversion of glucose and glycogen allows for storage of glucose as glycogen when glucose levels are high and the rapid, reverse conversion of glycogen to glucose when energy demands are high such as during physical activity. Primarily found in the liver and muscle, glycogen is a branched polymer consisting of glucose chains formed through α-1,4 linkages with branches inserted through α-1,6 linkages occurring every several glucose units. Metabolic abnormalities of glycogen metabolism, referred to as glycogen storage diseases, include Von Gierke, Pompe and Cori diseases. AkrivisBio's Glycogen Assay II provides a simple, sensitive method of measuring glycogen levels in biological materials. In the assay, glycogen is hydrolyzed to glucose, which is oxidized to form NADH, used to convert a nearly colorless tetrazolium to an intensely colored formazan absorbing at 450 nm with a sensitivity below 2 µg/ mg tissue or cell pellet.

Assay Principle:

1 – Glycogen is hydrolyzed to glucose by amyloglucosidase
2 – glucose is oxidized by glucose dehydrogenase forming NADH
3 – NADH transfers its electron to WST8 (tetrazolium) forming the formazan with absorbance at 450 nm.

Assay Components:

Hydrolysis Buffer	25 ml	NM	MA-0136A
Development Buffer	25 ml	WM	MA-0136B
Amyloglucosidase	Lyoph	Blue	MA-0136C
Glucose Dehydrogenase	Lyoph	Green	MA-0136D
WST8 Reagent	Lyoph	Red	MA-0136E
Glycogen Standard	500 µl	Yellow	MA-0136F

Storage and Handling:

Store kit at -20°C. Warm buffers to room temperature before use. Centrifuge all small vials for a few seconds, prior to opening.
Hydrolysis Buffer, Development Buffer: Ready to use as supplied. Bring to room temperature before use. Store at 4°C.
Amyloglucosidase: Reconstitute with 220 µl Hydrolysis Buffer. If the assay is to be used repeatedly over a period of time, aliquot to convenient portions and store at -20°C to avoid repeated freeze-thaw cycles. Keep on ice while in use.
Glucose Dehydrogenase: Reconstitute with 220 µl DI H_2O. If the assay is to be used repeatedly over a period of time, aliquot to convenient portions and store at -20°C to avoid repeated freeze-thaw cycles. Keep on ice while in use.
WST-8 Reagent: Reconstitute with 220 µl DI H_2O. Store at -20°C.
Glycogen Standard: 80 µg/ml. Ready to use as supplied. Store at -20°C.

Assay Protocol:

1. Standard Curve:. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl of 80 µg/ml Glycogen Standard into a series of wells in a 96 well plate giving 0 - 0.4 - 0.8 - 1.2 - 1.6 - 2 µg/well. Adjust al well volumes to 50 µl with Hydrolysis Buffer.
2. Sample Preparation: Homogenize tissue (10 mg) or cells (1 x 10^6) quickly with 100 µl DI H_2O on ice. Boil homogenates for 5-10 min to inactivate enzymes. Centrifuge at 16,000 X g for 5 min to remove particulate material. Carefully transfer the clear supernatant to a fresh tube and note the total volume. Transfer portion to be analyzed (5-50 µl) to wells in a 96 well plate and bring all well volumes to 50 µl with Hydrolysis Buffer.
Note: Glucose in samples generates significant background. Run such samples in duplicate, using the paired sample as a background control well.

3. Hydrolysis: Add 2 µl of amyloglucosidase to all Standard and sample wells **(NOT to background control wells)**. Incubate at RT for 30 minutes.

4. Initiate Color Development: Each well requires 50 µl of Reaction Mix. Prepare a sufficient amount for the number of sample and standard wells run containing:

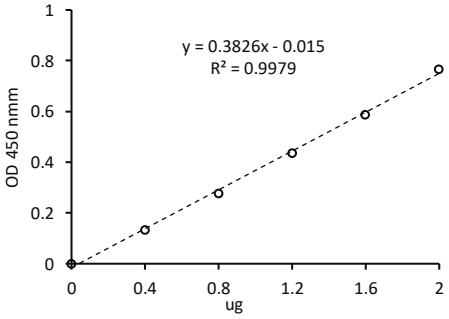
	Reaction Mix	Background Control Mix
Development Buffer	44 µl	46 µl
Glucose dehydrogenase	2 µl	2 µl
WST-8 Reagent	2 µl	2 µl

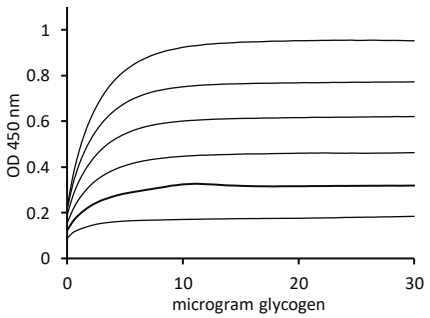
Add 50 µl of the Reaction Mix to sample and standard wells and Background Control Mix to background control wells.

5. Measurement: Monitor color development at 450 nm at room temperature for 30 minutes in a plate reader.
Note: It is important that all sample readings are within the standard curve range. For any samples outside of that range, dilute appropriately and rerun.

6. Typical Results:

µg Standard	Standard Raw Values OD	Background Corrected Values OD
0	0.1840	0
0.4	0.3187	0.1347
0.8	0.4626	0.2786
1.2	0.6205	0.4365
1.6	0.7719	0.5879
2.0.	0.9518	0.7678





7. Calculation: Subtract 0 Glycogen Standard reading from all standard readings. Plot the Glycogen Standard curve. Determine the slope of the standard curve. If background control wells have been run, subtract those values from the paired sample readings to obtain corrected absorbance readings for all samples. Divide the corrected sample readings by the slope of the standard curve to obtain µg sample glycogen in the wells. Convert to glycogen in original samples by:

A. Divide µg glycogen in well by µl sample applied to well to get µg glycogen per µl sample.
B. Multiply the µg glycogen per µl sample by the total volume determined in step 2 above = total glycogen obtained from sample
C. Divide total glycogen obtained from sample by mg tissue (# of cells, etc.) to obtain glycogen per mg tissue (# of cells etc.)

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