APExBIO
An Apoptosis and Epigenetics Company

Product Information

Glycogen Colorimetric Assay Kit II

I. Kit Contents:

Components	K2144-100 100 assays	Cap Color	Part Number
Glycogen Hydrolysis Buffer	25 ml	NM	K2144-C-1
Glycogen Development Buffer	25 ml	WM	K2144-C-2
Hydrolysis Enzyme Mix (Lyophilized)	1 vial	Blue	K2144-C-3
Development Enzyme Mix (Lyophilized)	1 vial	Green	K2144-C-4
Probe (Lyophilized)	1 vial	Red	K2144-C-5
Glycogen Standard (2 mg/ml)	100 µl	Yellow	K2144-C-6

II. Introduction:

Glycogen is a multibranched polysaccharide of glucose in α-1,4 linkage with branching via α-1,6 linkage and serves as a form of energy storage in animals. Glycogen is the main storage form of glucose in body and is mainly synthesized in the liver and muscle. Abnormal ability to utilize glycogen is found in several genetic glycogen storage diseases and diabetes.

The Glycogen Colorimetric Assay Kit II provides a simple, fast and convenient way for detection of glycogen levels in various biological samples based on colorimetric method. In the assay, glycogen is hydrolyzed to glucose, which is oxidized to produce an intermediate that reduces a colorless Probe to a colored product with strong absorbance at 450 nm. The assay is suited for detection glycogen levels in samples that contain reducing substances, which may interfere with the oxidase-based assays The kit can detect less than 4 µg/ml of glycogen in samples and is suited for high-throughput assay.

III. Application:

Measurement of Glycogen in various tissues.

Analysis of metabolism and cell signaling.

III. Sample Type:

Animal tissues: Liver, Muscle etc.

Cell culture: Adherent or suspension cells.

V. User Supplied Reagents and Equipment:

96-well clear plate with flat bottom.

Multi-well spectrophotometer (ELISA reader).

VI. Storage and Handling:

Store kit at -20℃, protected from light. Warm Assay Buffers to room temperature before use. Briefly centrifuge small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

Hydrolysis Enzyme Mix: Reconstitute with 220 µl Glycogen Hydrolysis Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20℃. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.

Development Enzyme Mix: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20℃. Keep on ice while in use. Use within two months.

Probe: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Stable for 2 months at -20℃.

VIII. Glycogen Assay Protocol:

1. Sample Preparation: Tissue (10 mg) or cells (1 x 10^6) should be rapidly homogenized with 100 µl ice cold Glycogen Hydrolysis Buffer for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1 - 50 µl samples (~ 50 µg) into a 96 well plate and bring the volume to 50 µl with Glycogen Hydrolysis Buffer.

Notes:

a. For unknown samples, we suggest testing several doses of samples to ensure the readings are within the standard curve range.

b. Glucose in samples will generate background. If your sample has significant amount of glucose, a sample background control is required.

2. Standard Curve Preparation: Dilute Glycogen Standard to 0.2 mg/ml (0.2 µg/µl) by adding 10 µl of 2 mg/ml Glycogen Standard to 90 µl dH_2O, mix well. Add 0, 2, 4, 6, 8 and 10 µl of 0.2 mg/ml Glycogen Standard into series of wells in 96 well plate to generate 0, 0.4, 0.8, 1.2, 1.6 and 2 µg/well Glycogen Standard. Adjust volume to 50 µl per well with Glycogen Hydrolysis Buffer.

3. Hydrolysis: Add 2 µl of Hydrolysis Enzyme Mix to Standard and samples, mix well. Incubate at room temperature for 30 minutes.

Note: Don’t add Hydrolysis Enzyme Mix to the sample background control.

4. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix
Glycogen Development Buffer	44 µl	46 µl
Development Enzyme Mix	2 µl	2 µl
Probe	2 µl	2 µl

Add 48 µl of the Reaction Mix to each well containing the Standard and samples and 50 µl of Background Control Mix to background control well.

5. Measurement: Incubate at room temperature for 30 minutes. Measure OD 450 nm with a microplate reader.

6. Calculation: Subtract 0 Glycogen Standard reading from all readings. Plot the Glycogen Standard curve. If background control reading is significant, subtract the background control reading from sample reading. Apply the corrected sample reading to the Glycogen Standard curve to get B µg of Glycogen in the samples.

Sample Glycogen Concentration (C) = B/V x Dilution Factor = µg/µl = mg/ml

Where: B is the Glycogen amount from Standard Curve (µg).

V is the sample volume used in the reaction well (µl).

Sample glycogen concentration can also be expressed in µg/mg of sample or other desired method.

Glycogen molecular weight ~ 10^5 - 10^7 g/mol.

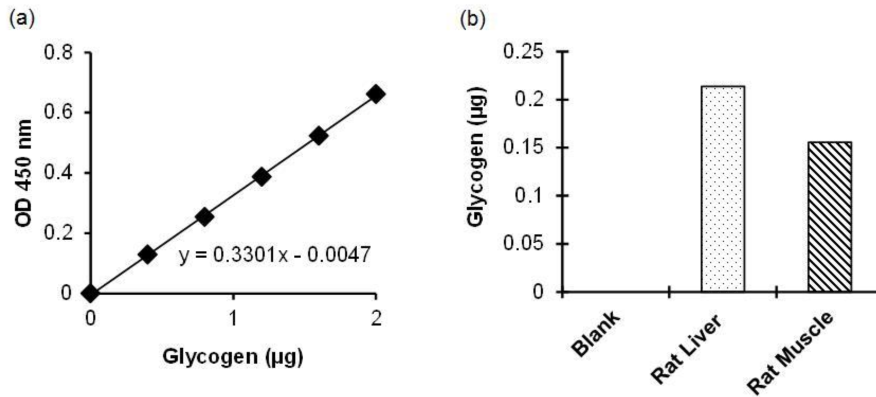

Figure. Glycogen Standard curve (a). Measurement of glycogen levels in rat Liver (20 µg) and rat muscle (40 µg). Assays were performed following Kit protocol.

Frequently Asked Questions

1. How should different dilutions of the sample be tested with this kit? Should the volumes be different in the well for each dilution?

Samples can be diluted over a range, for example, 1:10 or 1:50 or 1:100 or as required in a separate tube and then add 5 µl for all dilutions, 10ul for all dilutions and so on into the wells. Then fill up volume to 50 µl. This can be tested with one or two samples to optimize the dilution and the volume needed. If there is glucose in the sample, this can create background. A parallel background control can be used to subtract this value.

2. Why is there only 48 µl reaction mix for samples and standards where as there is 50 µl for the background?

The standard and the samples have 2 µl of the hydrolysis enzyme mix (step 3, section VIII). This is why there is 48 µl total reaction mix for the standards and the samples where as there is 50 µl reaction mix for the background control.

3. Can frozen samples be used with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

4. Is it possible to use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

5. Is it essential to make a standard curve for every expt, or is one curve/kit enough?

Yes, it is strongly recommended to do the standards every time you do the expt. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

6. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature (-20℃) and in appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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