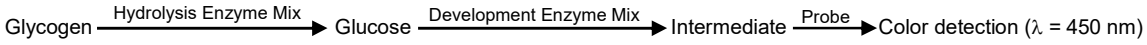
Glycogen Colorimetric Assay Kit II

(Catalog # BN00872; 100 assays; Store at -20°C)

I. Introduction:

Glycogen serves as the main carbohydrate storage in animals and can be converted to glucose readily. It is primarily found in the liver and muscle tissues. Glycogen is a branched biopolymer comprising of α-1,4 linkage with α-1,6 linkages occurring every 8-10 glucose units along the backbone. Abnormal ability to utilize glycogen is found in diabetes and in several genetic glycogen storage diseases. Assay Genie's Glycogen Assay kit II provides a simple, fast and robust way to measure Glycogen levels in various biological samples. In this assay, Glycogen is hydrolyzed into glucose, which is oxidized to form an intermediate that reduces a colorless Probe to a colored product with strong absorbance at 450 nm. This high-throughput suitable assay kit can detect less than 4 µg/ml of Glycogen in samples.



II. 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.

IV. Kit Contents:

Components	BN00872	Cap Code	Part Number
Glycogen Hydrolysis Buffer	25 ml	NM	BN00872-1
Glycogen Development Buffer	25 ml	WM	BN00872-2
Hydrolysis Enzyme Mix (Lyophilized)	1 vial	Blue	BN00872-3
Development Enzyme Mix (Lyophilized)	1 vial	Green	BN00872-4
Probe (Lyophilized)	1 vial	Red	BN00872-5
Glycogen Standard (2 mg/ml)	100 µl	Yellow	BN00872-6

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°C, 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°C. 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°C. 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°C.

VIII. Glycogen Assay Protocol:

1. **Sample Preparation:** Tissue (10 mg) or cells (1 x 10^6) should be rapidly homogenized with 200 µl ddH_2O for 10 minutes on ice. Boil the homogenates for 10 min to inactivate enzymes. Centrifuge at 18000 rpm for 10 min and remove insoluble material. Collect the supernatant. Supernatant is ready to be assayed. 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_{450nm} 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.

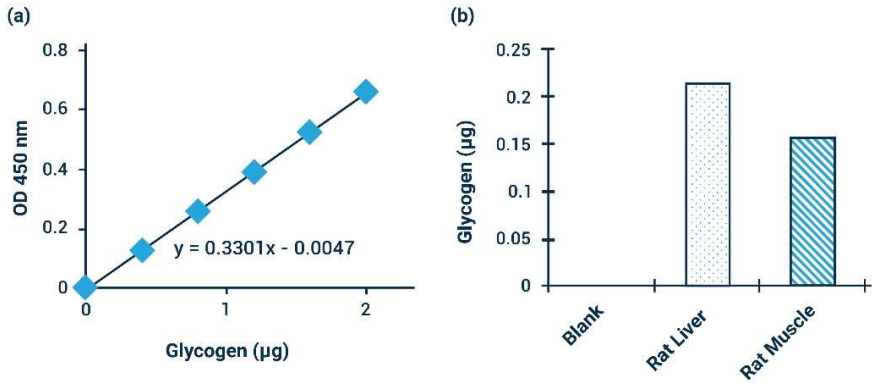


Figure. Glycogen Standard curve (a). Measurement of glycogen levels in rat Liver (20 µg) and rat muscle (40 µg). Assays were performed following Kit protocol.

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