

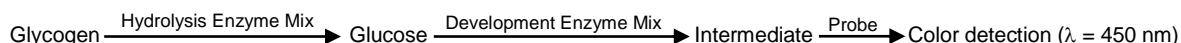
# Glycogen Colorimetric Assay Kit II

rev. 11/17

(Catalog # K648-100; 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  $\alpha$ -1,4 linkage with  $\alpha$ -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. Biovision'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  $\mu$ g/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	K648-100	Cap Code	Part Number
Glycogen Hydrolysis Buffer	25 ml	NM	K648-100-1
Glycogen Development Buffer	25 ml	WM	K648-100-2
Hydrolysis Enzyme Mix (Lyophilized)	1 vial	Blue	K648-100-3
Development Enzyme Mix (Lyophilized)	1 vial	Green	K648-100-4
Probe (Lyophilized)	1 vial	Red	K648-100-5
Glycogen Standard (2 mg/ml)	100 $\mu$ l	Yellow	K648-100-6

## 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  $\mu$ l 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  $\mu$ l dH<sub>2</sub>O. 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  $\mu$ l dH<sub>2</sub>O. 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 ( $1 \times 10^6$ ) should be rapidly homogenized with 200  $\mu$ l ddH<sub>2</sub>O 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  $\mu$ l samples (~50  $\mu$ g) into a 96 well plate and bring the volume to 50  $\mu$ l with Glycogen Hydrolysis Buffer.

### Notes:

- For unknown samples, we suggest testing several doses of samples to ensure the readings are within the standard curve range.
  - 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  $\mu$ g/ $\mu$ l) by adding 10  $\mu$ l of 2 mg/ml Glycogen Standard to 90  $\mu$ l dH<sub>2</sub>O, mix well. Add 0, 2, 4, 6, 8 and 10  $\mu$ l 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  $\mu$ g/well Glycogen Standard. Adjust volume to 50  $\mu$ l per well with Glycogen Hydrolysis Buffer.
  - 3. Hydrolysis:** Add 2  $\mu$ l 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  $\mu$ l Reaction Mix containing:

	Reaction Mix	Background Control Mix
Glycogen Development Buffer	44 $\mu$ l	46 $\mu$ l
Development Enzyme Mix	2 $\mu$ l	2 $\mu$ l
Probe	2 $\mu$ l	2 $\mu$ l

Add 48  $\mu$ l of the Reaction Mix to each well containing the Standard and samples and 50  $\mu$ l of Background Control Mix to background control well.

**5. Measurement:** Incubate at room temperature for 30 minutes. Measure OD<sub>450nm</sub> 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  $\mu$ g of Glycogen in the samples.

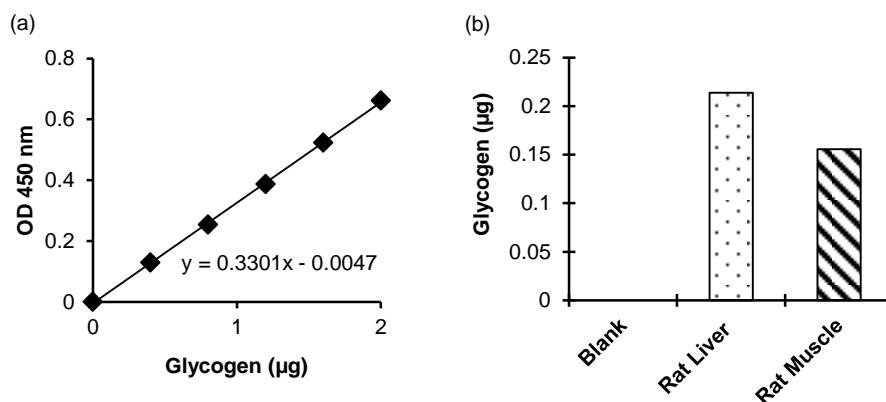
$$\text{Sample Glycogen Concentration (C)} = \text{B/V} \times \text{Dilution Factor} = \mu\text{g}/\mu\text{l} = \text{mg/ml}$$

Where: **B** is the Glycogen amount from Standard Curve ( $\mu$ g).

**V** is the sample volume used in the reaction well ( $\mu$ l).

Sample glycogen concentration can also be expressed in  $\mu$ g/mg of sample or other desired method.

Glycogen molecular weight  $\sim 10^5$ - $10^7$  g/mol.



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

#### IX. RELATED PRODUCTS:

Glycogen Assay Kit  
 Glucose Assay kit  
 Glucose Uptake Colorimetric Assay Kit  
 Glucose Uptake Fluorometric Assay Kit  
 Glucose Dehydrogenase Activity Assay Kit  
 Glucose-6-Phosphate Dehydrogenase Assay Kit

PicoProbe™ Glucose-6-Phosphate Assay Kit  
 Glucose and Sucrose Assay Kit  
 Maltose and Glucose Assay Kit  
 NADP/NADPH Quantification Kit  
 NAD/NADH Quantification Kit

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