

Glycogen Assay Kit

(Catalog #K646-100; 100 assays; Store at -20°C)

I. Introduction:

Glycogen is the primary short term energy storage molecule in animals, synthesized primarily in the liver and muscle. Glycogen is a branched glucose polymer, in α -1,4 linkage, with branching via α -1,6 linkage. Abnormal ability to utilize glycogen is found in diabetes and in several genetic glycogen storage diseases. The BioVision Kit is an easy and convenient assay to measure glycogen levels in biological samples. In the assay, glucoamylase hydrolyzes the glycogen to glucose which is then specifically oxidized to produce a product that reacts with OxiRed probe to generate color ($\lambda_{\max} = 570 \text{ nm}$) and fluorescence (Ex 535/Em 587). The assay can detect glycogen 0.0004 to 2 mg/ml.

II. Kit Contents:

Components	K646-100	Cap Code	Part Number
Hydrolysis Buffer	25 ml	NM	K646-100-1
Development Buffer	25 ml	WM	K646-100-2
OxiRed Probe	lyophilized	Red	K646-100-3
DMSO (anhydrous)	0.4 ml	Brown	K646-100-4
Hydrolysis Enzyme Mix	1 vial	Blue	K646-100-5
Development Enzyme Mix	lyophilized	Green	K646-100-6
Glycogen Standard (2.0 mg/ml)	100 μ l	Yellow	K646-100-7

III. Storage and Handling:

Store kit at -20°C, protect from light and moisture. Warm Glycogen Assay Buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

IV. Reagent Preparation and Storage Conditions:

OxiRed Probe: Dissolve with 220 μ l of DMSO (provided, need to warm up >18°C to become liquid) before use. Mix well, store at -20°C, protect from light and moisture.

Hydrolysis Enzyme Mix: Dissolve with 220 μ l Hydrolysis Buffer. Vortex gently to dissolve. Keep on ice. Store at -20°C. Stable for at least two months.

Development Enzyme Mix: Dissolve with 220 μ l Development Buffer. Vortex gently to dissolve. Keep on ice. Store at -20°C. Stable for two months.

V. Glycogen Assay Protocol:

1. Standard Curve Preparations:

Colorimetric Assay: Dilute the Glycogen Standard to 0.2 mg/ml by adding 10 μ l of the Standard to 90 μ l of distilled water, mix well. Add 0, 2, 4, 6, 8, 10 μ l to a series of wells. Adjust volume to 50 μ l/well with Hydrolysis Buffer to generate 0, 0.4, 0.8, 1.2, 1.6 and 2.0 μ g per well of the Glycogen Standard.

Fluorometric Assay: Dilute the Glycogen Standard to 0.02 mg/ml by adding 10 μ l of the Standard to 990 μ l of distilled water, mix well. Add 0, 2, 4, 6, 8, 10 μ l to a series of wells. Adjust volume to 50 μ l/well with Hydrolysis Buffer to generate 0, 0.04, 0.08, 0.12, 0.16 and 0.2 μ g per well of the Glycogen Standard.

2. **Sample Preparation*:** Liquid samples can be assayed directly. For tissue or cells, homogenize 10^5 cells or 10 mg tissue with 200 μ l dH₂O on ice. Boiling the homogenates for 5 min to inactivate enzymes. Spin the boiled samples at 13000rpm for 5 min to remove insoluble material, the supernatant is ready for assay. Add up to 50 μ l of sample or buffer (blank) to test wells. Adjust the volume to 50 μ l with Hydrolysis Buffer. For unknown samples, we suggest testing several doses of your sample to ensure the readings are within the standard curve.

* **Notes:** A. Glycogen can be metabolized very rapidly in some tissues after death (within a minute). Therefore, special cares must be taken to minimize glycogen consumption when take tissue sample, such as, frozen samples immediately, keep cold while working.

B. There are a variety of methods for extraction of glycogen from tissues¹⁻⁴ depending upon the type of tissue or type of information desired, such as using 30%KOH extraction/ethanol precipitation method to remove glucose background⁵, or measure glycogen molecular weight distribution¹, etc.

3. Hydrolysis**:

Hydrolysis Enzyme Mix

Colorimetric

2 μ l

Fluorometric

1 μ l

Add Hydrolysis Enzyme Mix into Standard and samples, mix well, incubate for 30 minutes at room temperature.

****Note:** Glucose generates background readings (such as, culture medium, ect.). If glucose is present in your sample, you may do a glucose control without the addition of hydrolysis enzyme to determine the level of glucose background in your sample. The glucose background can then be subtracted from glycogen readings.

4. Development:

Mix enough reagents for the number of samples and standards to be performed: For each well, prepare a total 50 μ l Reaction Mix.

Development Buffer

Colorimetric

46 μ l

Fluorometric

48.7 μ l

Development Enzyme Mix

2 μ l

1.0 μ l

OxiRed Probe

2 μ l

0.3 μ l

Add 50 μ l of the Reaction Mix to each well containing Glycogen Standard or samples.

Incubate at room temperature for 30 minutes, protect from light.

6. Measure colorimetrically (OD at 570 nm) or fluorometrically (Ex/Em 535/587 nm).

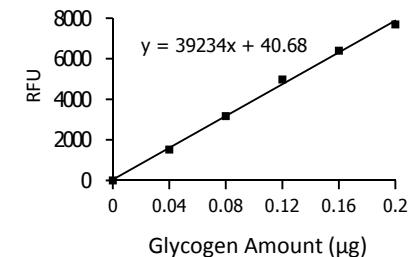
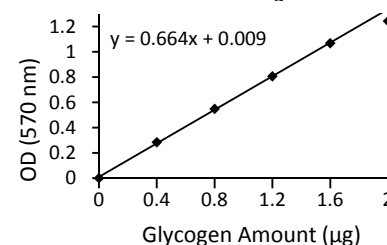
7. **Calculation:** Correct background by subtracting the 0 glycogen control from all sample readings (Note: The background can be significant and must be subtracted). Plot standard curve μ g/well vs. standard readings. Apply sample readings to the standard curve to get the amount of glycogen in the sample wells. The glycogen concentration in the test samples:

$$C = Ay/Sv \text{ (}\mu\text{g}/\mu\text{l, or mg/ml)}$$

Where: Ay is the amount of glycogen (μ g) in your sample from the standard curve.
Sv is the sample volume (μ l) added to the sample well.

Glycogen molecular size: $\sim 60,000$ glucose molecules (MW $\sim 10^6$ - 10^7 daltons).

Glucose Molecular Weight: 180.16.



Glycogen Standard Curve: Assays were performed following the kit protocol.

VI. 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. Int 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.