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 (λ_{max} = 570 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	0.2 ml	Red	K646-100-3A
Hydrolysis Enzyme Mix	lyophilized	Blue	K646-100-5
Development Enzyme Mix	lyophilized	Green	K646-100-6
Glycogen Standard (2.0 mg/ml)	100 µl	Yellow	K646-100-7

III. Storage and Handling:

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

IV. Reagent Preparation and Storage Conditions:

OxiRed Probe: Ready to use as supplied. Warm to >18°C to melt frozen DMSO before use. Mix well, store at –20°C, protect from light and moisture. Use within 2 months.

Hydrolysis Enzyme Mix, Development Enzyme Mix: Dissolve with 220 µl Hydrolysis Buffer. Vortex tubes gently to dissolve. Keep on ice. Store at –20°C. Reagents are stable for at least two months.

V. Glycogen Assay Protocol:

1. Standard Curve Preparations:

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

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

2. Sample Preparation*:

Liquid samples can be assayed directly. For tissue or cells, homogenize 10^6 cells or 10 mg tissue with 200 µl dH_2O on ice. Boil the homogenates for 5 min to inactivate enzymes. Spin the boiled samples at 13000 rpm for 5 min to remove insoluble material; the supernatant is ready for assay.

Add up to 50 µl of sample or buffer (blank) to test wells. Adjust the volume to 50 µl 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 care must be taken to minimize glycogen loss when taking tissue samples, such as freezing samples immediately and keeping cold while working.

B. There are a variety of methods for extraction of glycogen from tissues[1-4] depending upon the type of tissue or type of information desired. You should consult the literature to determine the best method for your purposes.

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3. **Hydrolysis**:**

	Colorimetric	Fluorometric
Hydrolysis Enzyme Mix	2 µl	1µl

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

****Note:** Glucose generates background readings. 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 µl Reaction Mix.

	Colorimetric	Fluorometric
Development Buffer	46 µl	48.7 µl
Development Enzyme Mix	2 µl	1.0 µl
OxiRed Probe	2 µl	0.3 µl

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

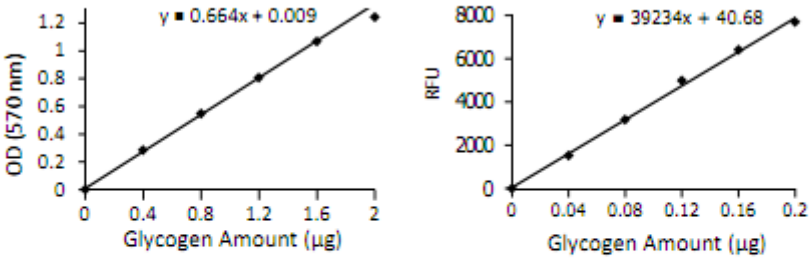
5. Incubate at room temperature for 30 min, protect from light.
6. Measure color (OD at 570 nm) or fluorescence (Ex/Em 535/587 nm).
7. **Calculation:** Correct background by subtracting the 0 glycogen control from all sample readings (Note: The background can be significant and must be subtracted). Plot standard curve µg/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 (µg/µl, or mg/ml)

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

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

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. Intl 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.

Sample Preparation:

There are a variety of methods for extraction of glycogen from tissues depending upon a) the type of tissue the glycogen is to be extracted form and b) the type of information desired. The gentlest procedure is the method referred in reference 1, which maintains the molecular weight of the glycogen so that analysis of the molecular distribution is possible.

A rapid method useful for small tissue samples is detailed in reference 4. Basically a small sample of tissue is homogenized in 50 volumes of distilled water, diluted appropriately and immediately used in the assay. Since endogenous glucose will be a significant factor utilizing this method, a glucose background control must be conducted where the sample is directly placed in development buffer with development enzyme mix (without prior treatment with the hydrolysis reagents). If the sample will not be immediately assayed, it should be placed in a capped, vented microcentrifuge tube and boiled for 5 min to inactivate any enzyme activities present and stored at -20°C until assayed. Samples from high content tissues (liver, muscle) prepared in this way should have sufficient glycogen such that 5-25 µl aliquots will give a clearly measureable colorimetric signal. If the sample is from low content tissues, either take a large aliquot (50µl) for the colorimetric assay or a proportionally smaller aliquot (10-25µl) in the fluorometric assay.

Caveats:

1) in some tissues such as neural tissue, very rapid rates of anaerobic metabolism continue after death causing rapid declines in glucose to undetectable levels within a few seconds. Utilization of glycogen follows and large decreases in glycogen content are seen within less than a minute. Thus accurate measurement of glycogen in such tissues requires very rapid quenching of metabolic activity such as freeze clamp or immediate removal of tissue to liquid nitrogen followed by grinding in the liquid nitrogen and storage at -20 or -80°C until used.

2) In some samples i.e., Saccharomyces, the glycogen is distributed between soluble and insoluble pools. It is not clear that both pools are completely hydrolyzed

If the sample to be analyzed is sufficiently large (a few hundred milligrams to grams of tissue), a more quantitative method is as follows:

Take tissue or cells to a final content of 30-50% in 30% KOH. Heat to 100°C for 2 hours, cool and add 2 volumes of 95% ethanol. This will precipitate the crude glycogen. Centrifuge and collect the precipitate. Dissolve/suspend the precipitate in a minimal amount of distilled water and acidify to pH 3 with HCL (5N). Reprecipitate with 1 volume of ethanol. Repeat wash/acidification/precipitation 2 more times, then wash precipitate with ethanol and dry. This procedure removes the vast majority of the glucose background with minimal effect on the glycogen. The dried material can be weighed and dissolved/suspended in hydrolysis buffer for analysis.