Product Information

Glycogen Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2143-100 100 assays	Cap Color	Part Number
Hydrolysis Buffer	25 ml	NM	K2143-C-1
Development Buffer	25 ml	WM	K2143-C-2
OxiRed Probe	0.2ml	Red	K2143-C-3
Hydrolysis Enzyme Mix	lyophilized	Blue	K2143-C-4
Development Enzyme Mix	lyophilized	Green	K2143-C-5
Glycogen Standard (2.0 mg/ml)	100 µl	Yellow	K2143-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/Fluorometric Assay Kit provides a sensitive, fast and convenient way for accurate detection of glycogen levels in various biological samples based on colorimetric and fluorometric method. In the assay, glucoamylase hydrolyzes glycogen to glucose, which is subsequent specifically oxidized to generate a product that reacts with OxiRed probe to yield fluorescence (Ex 535/Em 587) and color (570 nm). The kit can detect 0.0004 to 2 mg/ml glycogen.

III. Application:

Measurement of Glycogen in various tissues/cells.

Analysis of metabolism and cell signaling in various cells.

III. Sample Type:

Animal tissues such as liver etc.

Cell culture: adherent or suspension cells.

V. User Supplied Reagents and Equipment:

96-well plate with flat bottom.

Multi-well spectrophotometer.

VI. Storage and Handling:

Store kit at -20°C, protected from light and moisture. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

Hydrolysis Buffer: Warm to room temperature before use. Store at -20°C or 4°C.

Development Buffer: Warm to room temperature before use. Store at -20°C or 4°C.

OxiRed Probe: Ready to use as supplied. Warm to room temperature to melt frozen DMSO before use. Mix well, store at -20°C. Protect from light and moisture. Use within 2 months.

Hydrolysis Enzyme Mix: Reconstitute with 220 µl of Hydrolysis Buffer. Vortex gently to dissolve. Keep on ice. Store at -20°C. Use within two months.

Development Enzyme Mix: Reconstitute with 220 µl of Development Buffer. Vortex gently to dissolve. Keep on ice. Store at -20°C. Reagents are stable for at least two months

VIII. Glycogen Assay Protocol:

1. 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 10 min. to inactivate enzymes. Spin the boiled samples at 18,000 x g for 10 min. to remove insoluble material; the supernatant is ready for assay. Add 2 - 50 µl samples to a 96-well plate. Adjust the volume to 50 µl/well with Hydrolysis Buffer.

Notes:

a. For unknown samples, we suggest performing a pilot experiment & testing different sample dilutions to ensure the readings are within the Standard Curve range.

b. Glycogen can be metabolized very rapidly in some tissues after death (within a min.), therefore special care must be taken to minimize glycogen loss when taking tissue samples, such as freezing samples immediately and keeping cold while working.

c. For samples having glucose background, prepare parallel well(s) containing same amount of sample as in the test well as background control (see section 3).

d. Endogenous compounds may interfere with the reaction. To ensure accurate determination of Glycogen in the test samples, we recommend spiking samples with a known amount of Standard (0.8 µg).

e. There are varieties of methods for extraction of glycogen from tissues1-4 depending upon the type of tissue or type of information desired. We strongly recommend consulting the literature to determine the best method for your purposes. However, for convenience a few methods taken from literature are described on page 3.

2. Standard Curve Preparation: For 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. For 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.

3. Hydrolysis: Add Hydrolysis Enzyme mix to Standards and samples and mix well.

	Colorimetric	Fluorometric
Hydrolysis Enzyme Mix	2 µl	1 µl

Incubate for 30 min. 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. Reaction Mix: 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 the Glycogen Standard or samples, mix well.

5. Measurement: Incubate the reaction for 30 min. at room temperature, protected from light. Measure absorbance (OD 570 nm) or fluorescence (Ex/Em = 535/587 nm).

6. Calculation: Correct background by subtracting the 0 glycogen Standard from all readings (Note: The background can be significant and must be subtracted). Plot Glycogen Standard Curve. Apply sample readings to the standard curve to get B µg of glycogen in the sample wells.

Sample Glycogen concentration (C) = B/V X D µg/µl

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

V is the sample volume added into the reaction well (µl).

D is the sample dilution factor.

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, Glycogen amount in sample well (B) =($OD_{sample\ (corrected)}$)/ ($OD_{sample + Glycogen\ Std(corrected)}$ - $OD_{sample\ (corrected)}$))* Glycogen Spike (nmol).

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

Glucose Molecular Weight: 180.16.

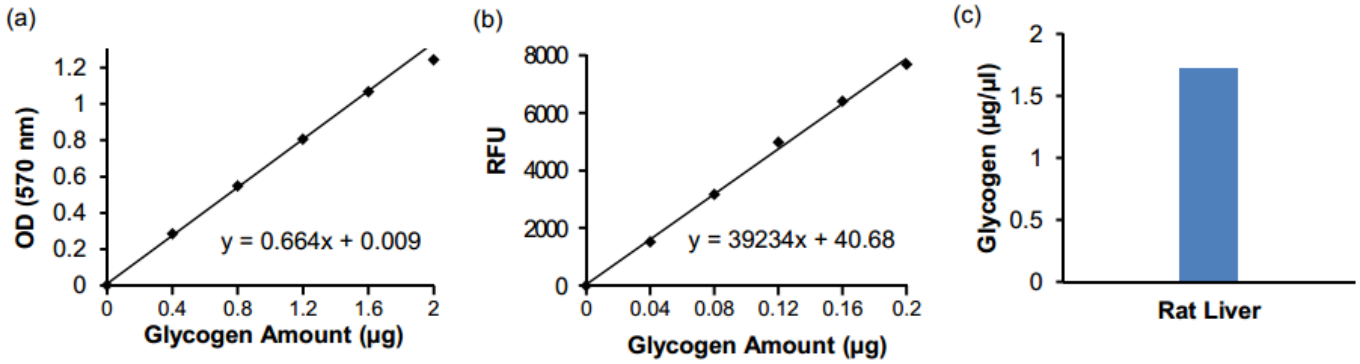

Figure: Glycogen Standard Curve: colorimetric (a), fluorometric (b). c) Representative Glycogen Standard Curve in the presence of rat liver lysate. Sample of rat liver was homogenized with deionized water (0.05 mg liver/µl water). Homogenate was boiled for 10 min., centrifuged for 10 min. at 18000 x g and supernatant was collected. Samples (5 µl of 10 times diluted) were spiked with glycogen (0.8 µg) and assayed according to the protocol. Assays were performed following the kit protocol.

Frequently Asked Questions:

1. Where is the glycogen standard from? Is it chemically synthesized?

The glycogen standard is from an animal-derived source.

2. Is there any glucose in the glycogen standard?

There should not be any glucose in the glycogen standard. Glucose in the samples can generate background in this assay.

3. Please explain the mechanism of detection. Is it the same as the Chan and Exton paper titled "A rapid method for the determination of glycogen content and radioactivity in small quantities of tissue or isolated hepatocytes " ?

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 ($OD_{570\ nm}$) and fluorescence (Ex/Em = 535/587 nm). The assay can detect glycogen 0.0004 to 2 mg/ml. Our assay is non-radioactive. We do not precipitate samples on filter paper to assay glycogen as was done in the Chan and Exton paper. Based on the sample type, different methods of glycogen extraction are recommended on the datasheet.

4. 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.

5. Can this kit be used with samples like bacteria, plants, drosophila, yeast etc?

We have optimized the kit with mammalian samples. However, theoretically these kits should work with samples from multiple species/sources. Since the optimal conditions depend on the sample type, the protocol has to be be adapted to fit the samples for efficient results. Please refer to this kits citations to see what kind of samples have been used with this kit other than mammalian samples.

6. How can mg of glycogen be converted to Molar concentration? How else can the data be reported?

mg of glycogen can be converted to mmoles of glycogen using molecular weight of glycogen (since the exact weight of glycogen in a sample can vary, it is a more precise way to report mg of glycogen). Instead of the per ml glycogen, mg of tissue used per well can be calculated based on the starting amount and the volume added to each well. In this way then mg of glycogen /mg tissue can be reported.

7. 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.

8. What would be a good positive control sample tissue for glygogen in rat?

Liver is the main site for glycogen storage. Hence rat liver was used for our data on the datasheet and you can use this too.

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.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com