



Glycogen Assay Kit

Item No. 700480

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results store kit at -20°C or remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
700481	Glycogen Standard	2 vials	-20°C
700482	Glycogen Assay Buffer (5X)	1 vial/14 ml	-20°C
700483	Glycogen Hydrolysis Enzyme	2 vials	-20°C
700484	Glycogen Hydrolysis Buffer	1 vial/10 ml	-20°C
700485	Glycogen Enzyme Mixture	2 vials	-20°C
700486	Glycogen Fluorometric Detector	2 vials	-20°C
700001	DMSO Assay Reagent	1 vial/1 ml	RT
400017	96-Well Solid Plate (black)	1 plate	RT
400012	96-Well Cover Sheet	1 cover	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888
Fax: 734-971-3641
Email: techserv@caymanchem.com
Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the ability to measure fluorescence using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm
2. Adjustable pipettes and a multichannel or repeating pipette
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

Background

Glycogen is a polysaccharide that is the principal storage form of glucose in animal and human cells. Structurally, glycogen is a highly branched glucose polymer made of $\alpha(1,4)$ linkages with $\alpha(1,6)$ linkages occurring every 8-10 glucose units along the backbone. Glycogen is made primarily by the liver and muscles, but it can also be made by glycogenesis within the brain and stomach. Glycogen is found in the form of granules in the cytosol of the cells and plays an important role in the glucose cycle.

Liver hepatocytes have the largest concentration of glycogen - up to 10% of the fresh weight (100-120 g in adults) after a meal.¹ Only glycogen which is stored in the liver can be made accessible to the other organs. In muscles, glycogen is found in much lower concentrations (1-2% of the muscle mass), but the total amount surpasses that of the liver amount. Muscle cell glycogen seems to function as an immediate reserve source of available glucose for muscle cells, since it lacks the enzyme glucose-6-phosphatase, which is required to pass the glucose into the blood. This glucose can then be readily available during bursts of activity. Smaller amounts of glycogen can be found in kidneys, certain glial cells in the brain and white blood cells. The uterus also stores glycogen during pregnancy to nourish the embryo.^{2,3}

The most common disease involving abnormal glycogen metabolism is diabetes mellitus. Several inborn errors of metabolism caused by inherited genetic deficiencies of enzymes which are involved with glycogen synthesis or break down (glycogen storage diseases) include von Gierke's disease, Pompe disease, and McArdle's disease.⁴

About This Assay

Cayman's Glycogen Assay provides a simple, reproducible, and sensitive tool for assaying glycogen from tissue. Glycogen is hydrolyzed by amyloglucosidase to form β -D-glucose, which is then specifically oxidized by glucose oxidase to form hydrogen peroxide. Hydrogen peroxide, in the presence of horseradish peroxidase, reacts with 10-acetyl-3,7-dihydroxyphenoxazine (ADHP) in a 1:1 stoichiometry to generate the highly fluorescent product resorufin. Resorufin fluorescence is measured with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

Reagent Preparation

1. Glycogen Standard - (Item No. 700481)

Each vial contains a lyophilized powder of glycogen. Reconstitute the contents of the vial with 1 ml of diluted Assay Buffer to obtain a solution of 2 mg/ml glycogen. This will be used to prepare the standard curve (see **Standard Curve Preparation** on page 13). When stored at room temperature, the reconstituted standard is stable for two hours.

2. Glycogen Assay Buffer (5X) - (Item No. 700482)

The vial contains 14 ml of a concentrated phosphate buffer, pH 7.0. Thaw at room temperature and dilute the contents with 56 ml UltraPure water. Be certain to rinse the vial to remove any salts that may have precipitated. When stored at 4°C, the diluted Assay Buffer is stable for at least three months.

NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.

3. Glycogen Hydrolysis Enzyme - (Item No. 700483)

Each vial contains a lyophilized powder of amyloglucosidase. Reconstitute the contents of the vial with 3 ml of Glycogen Hydrolysis Buffer (Item No. 700484) and store on ice. One vial is enough to assay 60 wells. If additional wells are being utilized, then reconstitute the second vial. When stored at 4°C, the reconstituted solution is stable for ninety minutes.

4. Glycogen Hydrolysis Buffer - (Item No. 700484)

The vial contains 10 ml of 50 mM acetate buffer, pH 4.5. Thaw the vial at room temperature. The buffer is ready to use as supplied. Store any unused buffer at 4°C. The buffer is stable for two months at 4°C.

5. Glycogen Enzyme Mixture - (Item No. 700485)

Each vial contains a lyophilized Enzyme Mixture. Reconstitute the contents of the vial with 2.5 ml of diluted Assay Buffer and store on ice. This will be used to prepare the 'Developer' (see **Performing the Assay**, on page 14, for further instructions.) One vial is sufficient to assay 53 wells. Use the second vial if utilizing the entire plate. When stored at 4°C, the reconstituted Enzyme Mixture is stable for ninety minutes.

6. Glycogen Fluorometric Detector - (Item No. 700486)

Each vial contains a clear lyophilized powder of 10-acetyl-3,7-dihydroxyphenoxazine (ADHP). It will be used to prepare the 'Developer' (see **Performing the Assay**, on page 14 for instructions for reconstitution). Once reconstituted, ADHP is stable for 60 minutes. After 60 minutes, increased background fluorescence will occur. One vial is sufficient to assay 53 wells. Use the second vial if utilizing the entire plate.

7. DMSO Assay Reagent - (Item No. 700001)

The vial contains 1 ml of dimethylsulfoxide (DMSO). Thaw the vial at room temperature. The reagent is ready to use as supplied.

Sample Preparation

NOTE: Glycogen can be metabolized very rapidly after harvesting samples. In order to minimize glycogen consumption, it is suggested that the samples be "flash frozen" in liquid nitrogen immediately upon collection and keep all samples on ice while performing the assay. Storage of the samples for extended periods is not recommended. If you elect to store samples, glycogen concentrations may be lower than expected.

Tissue

1. Weigh the frozen tissue and then mince into small pieces.
2. Homogenize 350-400 mg of minced tissue in 2 ml of the diluted Assay Buffer containing protease inhibitors of choice (see page 19).
3. Centrifuge at 800 x g for 10 minutes at 4°C.
4. Transfer the supernatant to another tube. Store the supernatant on ice.
5. Typically, tissue samples require dilutions of at least 1:10 or greater. Dilute the samples using the diluted Assay Buffer before assaying.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. We suggest that each sample and standard be assayed at least in duplicate (triplicate is preferred). A typical layout of samples to be measured in duplicate is given below in Figure 1. We suggest you record the contents of each well on the template sheet provided (see page 22).

	1	2	3	4	5	6	7	8	9	10	11	12
A	(A)	(A)	(S1)	(S1)	(S5)	(S5)	(S9)	(S9)	(S13)	(S13)	(S17)	(S17)
B	(B)	(B)	(B1)	(B1)	(B5)	(B5)	(B9)	(B9)	(B13)	(B13)	(B17)	(B17)
C	(C)	(C)	(S2)	(S2)	(S6)	(S6)	(S10)	(S10)	(S14)	(S14)	(S18)	(S18)
D	(D)	(D)	(B2)	(B2)	(B6)	(B6)	(B10)	(B10)	(B14)	(B14)	(B18)	(B18)
E	(E)	(E)	(S3)	(S3)	(S7)	(S7)	(S11)	(S11)	(S15)	(S15)	(S19)	(S19)
F	(F)	(F)	(B3)	(B3)	(B7)	(B7)	(B11)	(B11)	(B15)	(B15)	(B19)	(B19)
G	(G)	(G)	(S4)	(S4)	(S8)	(S8)	(S12)	(S12)	(S16)	(S16)	(S20)	(S20)
H	(H)	(H)	(B4)	(B4)	(B8)	(B8)	(B12)	(B12)	(B16)	(B16)	(B20)	(B20)

A-H = Standards

S1-S20 = Sample wells

B1-B20 = Sample background wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 210 μl in all wells.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples be assayed at least in duplicate (triplicate is preferred), but it is the user's discretion to do so.
- The assay is performed at 37°C.
- Monitor the fluorescence with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

Standard Curve Preparation

Take eight clean test tubes or polystyrene tubes and label them A-H. Dilute 100 μl of the 2 mg/ml Glycogen Standard with 900 μl of diluted Assay Buffer to yield a 200 $\mu\text{g/ml}$ Glycogen Standard. Add the amount of 200 $\mu\text{g/ml}$ Glycogen Standard and diluted Assay Buffer to each tube as described in Table 1. We recommend that you store these diluted standards for no more than one hour.

Tube	200 $\mu\text{g/ml}$ Glycogen (μl)	diluted Assay Buffer (μl)	Glycogen ($\mu\text{g/ml}$)
A	0	1,000	0
B	12.5	987.5	2.5
C	25	975	5
D	50	950	10
E	75	925	15
F	100	900	20
G	150	850	30
H	200	800	40

Table 1. Preparation of Glycogen Standard Curve

Performing the Assay

1. **Standard Wells** - Add 10 μ l of standard (tubes A-H) per well in the designated wells on the plate (see **Sample plate format**, Figure 1, page 11).
2. **Sample Wells** - Add 10 μ l of the sample to at least two wells. To obtain reproducible results, the amount of glycogen added to the wells should fall within the range of the assay. When necessary, samples should be diluted with the diluted Assay Buffer.
3. **Sample Background Wells** - Add 10 μ l of the sample and 50 μ l of the Glycogen Hydrolysis Buffer (Item No. 700484) to at least two wells.
4. Add 50 μ l of the reconstituted Hydrolysis Enzyme Solution to all standard wells and sample wells. DO NOT add the reconstituted Hydrolysis Enzyme Solution to the sample background wells.
5. Cover the plate with the plate cover, and incubate for 30 minutes at 37°C.
6. Prepare the 'Developer' as follows within five to ten minutes prior to adding to the wells: Add 100 μ l of DMSO Assay Reagent (Item No. 700001) to one vial of the Glycogen Fluorometric Detector (Item No. 700486) and mix until dissolved. Then add 400 μ l of diluted Assay Buffer to the fluorometric detector and vortex. Add the following reagents together in the Enzyme Mixture vial as outlined in the Table below. This is enough 'Developer' for 53 wells. Prepare additional 'Developer' as needed. The prepared developer is stable for one hour.

Reagent	Amount
Fluorometric Detector	0.5 ml
Enzyme Mixture	2.5 ml
Diluted Assay Buffer	5 ml

7. Add 150 μ l of 'Developer' to all wells being used, including sample, sample background, and standards.
8. Cover and incubate for 15 minutes at 37°C.
9. Remove the plate cover and read using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

Calculations

1. Determine the average fluorescence of each standard, sample, and sample background.
2. Subtract the fluorescence value of standard A from itself and all other standards. This is the corrected fluorescence.
3. Plot the corrected fluorescence values (from step 2 above) of each standard as a function of the final concentration of glycogen from Table 1. See Figure 2, on page 18, for a typical standard curve.
4. Subtract the average fluorescence value of the sample background from the average fluorescence of the sample wells to yield the corrected sample fluorescence (CSF).
5. Calculate the glycogen concentration of the samples using the equation obtained from the linear regression of the standard curve substituting the corrected sample fluorescence (CSF) for each sample.

Glycogen concentration ($\mu\text{g/ml}$) =

$$\left[\frac{\text{CSF} - (\text{y-intercept})}{\text{Slope}} \right] \times \text{Sample dilution}$$

Performance Characteristics

Sensitivity:

The limit of detection for the assay is 2.5 $\mu\text{g/ml}$ ($\pm 0.5 \mu\text{g/ml}$) glycogen.

Precision:

When a series of ninety-six liver homogenate measurements were performed on the same day, the intra-assay coefficient of variation was 3.5%. When a series of ninety-six liver homogenate measurements were performed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 3.1%.

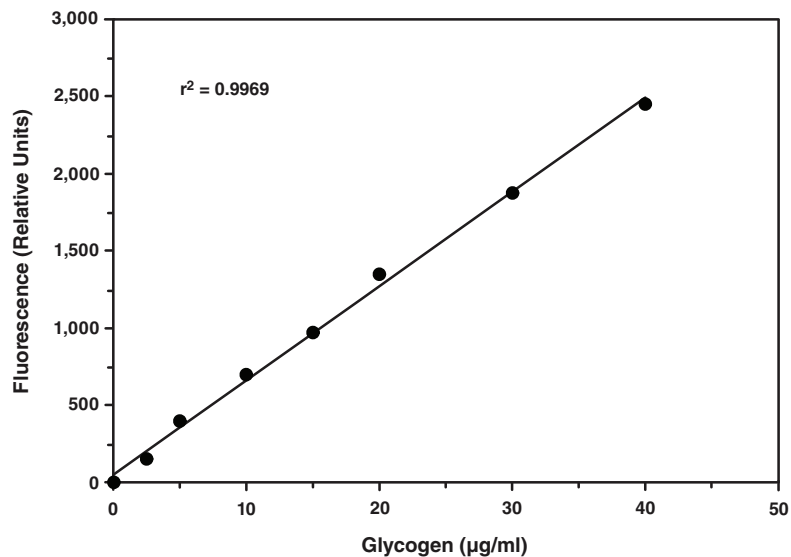


Figure 2. Glycogen standard curve

RESOURCES

Interferences

The following reagents were tested in the assay for interference in the assay:

	Reagent	Will Interfere (Yes or No)
Buffers	Tris	Yes
	HEPES	No
	Borate	Yes
	MES	No
Detergents	Polysorbate 20 (0.1%)	No
	Polysorbate 20 (1%)	Yes
	Triton X-100 (0.1%)	No
	Triton X-100 (1%)	No
Chelators	EDTA (1 mM)	No
	EGTA (1 mM)	Yes
Protease Inhibitors/ Enzymes	Trypsin (10 µg/ml)	No
	PMSF (200 µM)	No
	Leupeptin (10 µg/ml)	No
	Antipain (100 µg/ml)	No
	Chymostatin (10 µg/ml)	No
Solvents	Ethanol (5%)	Yes
	Methanol (5%)	No
	Dimethylsulfoxide (5%)	No
Others	BSA (0.1%)	No
	Glycerol (5%)	Yes

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No glycogen concentration was obtained above the corrected sample fluorescence (CF)	The glycogen concentration is too low to detect or does not contain glycogen	Re-assay the sample using a lower dilution
Glycogen concentration was above the highest point in the standard curve	The glycogen concentration was too high in the sample	Dilute samples with the diluted Assay Buffer and re-assay; <i>NOTE: Remember to account for the dilution factor when calculating glycogen concentration</i>
The fluorometer exhibited 'MAX' values for the wells	The GAIN setting is too high	Reduce the GAIN and re-read

References

- Horton, H.R. Glycogen storage diseases, Chapter 8, in Principles of biochemistry. Horton, H.R., Moran, L.A., Ochs, R.S. *et al.*, editors, editors, 9, Neil Patterson Publishers, Englewood Cliffs, NJ, 16-22 (1993).
- Burton, G.J., Watson, A.L., Hempstock, J., *et al.* Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy. *J. Clin. Endocrinol. Metab.* **87(6)**, 2954-2959 (2002).
- Hempstock, J., Cindrova-Davies, T., Jauniaux, E., *et al.* Endometrial glands as a source of nutrients, growth factors and cytokines during the first trimester of human pregnancy: A morphological and immunohistochemical study. *Reprod. Biol. Endocrinol.* **20(2)**, 58 (2004).
- Chen, Y.-T. and Burchell, A. Glycogen storage diseases, in The metabolic basis of inherited disease. Scriver, C.R., Beaudet, A.L., Sly, W.S. *et al.*, editors, editors, 7, McGraw-Hill, New York, 935-965 (1995).

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Warranty and Limitation of Remedy

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