Glycogen Assay Kit

Item No. 700480

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GENERAL INFORMATION

Materials Supplied

After opening the kit, store individual components 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
400014	96-Well Solid Plate (Colormetric Assay)	1 plate	RT
400012	96-Well Cover Sheet	1 ea	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.

This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

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 if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring fluorescence with excitation and emission wavelengths of 530-540 and 585-595 nm, respectively, or a plate reader capable of measuring absorbance at 570 nm.
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. A source of pure water; glass distilled water or pure water is acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*

INTRODUCTION

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 α(1,4) linkages with α(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 measuring glycogen in tissue/cells. 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. Alternatively, the absorbance can be measured at 570 nm.

This assay offers the option to measure absorbance or fluorescence. It is at the user's discretion to choose the mode of measurement (and corresponding standard curve preparation) that best fits their needs. When read fluorometrically, this assay has a dynamic range of 0.2-0.003 µg and a lower limit of quantification (LLOQ) of 0.003 µg. When read colorimetrically, the dynamic range is 2-0.031 µg, with an LLOQ of 0.031 µg.

PRE-ASSAY PREPARATION

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 Assay Buffer (1X) to obtain a solution of 2 mg/ml glycogen. It will be used to prepare the standard curve for either a fluorometric or colorimetric assay. (see **Standard Curve Preparation** on pages 13-14). 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 pure water. Be certain to rinse the vial to remove any salts that may have precipitated. When stored at 4°C, the Assay Buffer (1X) 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, 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 Assay Buffer (1X) and store on ice. This will be used to prepare the 'Developer' (see **Performing the Assay**, on page 15, 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 15 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. Fluorometric Detector is used in both colormetric and fluorometric formats.

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 Assay Buffer (1X) containing protease inhibitors of choice (see page 20).
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. Samples with high glycogen content, such as liver or muscle, should be diluted at least 1:50. Dilute the samples using Assay Buffer (1X) before assaying.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. It is suggested that each sample and standard be assayed at least in duplicate (triplicate is preferred) and that the contents of each well are recorded on the template sheet provided on page 22. A typical layout of samples to be measured in duplicate is shown in Figure 1.

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

1-8 = Standards
S1-S20 = Sample wells
B1-B20 = Sample background wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette 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 of the 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 at 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; or, monitor the absorbance at 570 nm.

NOTE: This assay can read using fluorescence or absorbance. You do not need to prepare both standard curves; choose the one that matches the format you will be using.

Standard Curve Preparation - Fluorometric

Dilute 50 µl of the 2 mg/ml Glycogen standard with 450 µl of diluted Assay Buffer to yield a 200 µg/ml Glycogen bulk standard. Label eight clean glass or polystyrene test tubes 1-8. Aliquot 450 µl of Glycogen Assay Buffer (1X) to tube 1. Aliquot 250 µl of Glycogen Assay Buffer (1X) to tubes 2-8. Transfer 50 µl of the bulk standard (200 µg/ml) to tube 1. Mix gently. Serially dilute the standard by removing 250 µl from tube 1 and placing it into tube 2. Mix gently. Next, remove 250 µl from tube 2 and place into tube 3. Mix gently. Repeat the process for tubes 4-7. Do not add any standard to tube 8. This tube is the zero-point, the lowest point of the standard curve.

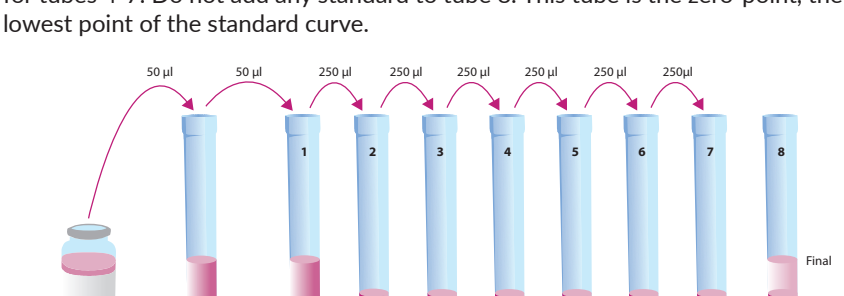


Figure 2. Preparation of glycogen standards - fluorometric

Standard Curve Preparation - Colorimetric

Label eight clean glass or polystyrene test tubes 1-8. Aliquot 450 µl of Glycogen Assay Buffer (1X) to tube 1. Aliquot 250 µl of Glycogen Assay Buffer (1X) to tubes 2-8. Transfer 50 µl of the reconstituted standard (2 mg/ml) to tube 1. Mix gently. Serially dilute the standard by removing 250 µl from tube 1 and placing it into tube 2. Mix gently. Next, remove 250 µl from tube 2 and place into tube 3. Mix gently. Repeat the process for tubes 4-7. Do not add any standard to tube 8. This tube is the zero-point, the lowest point of the standard curve.

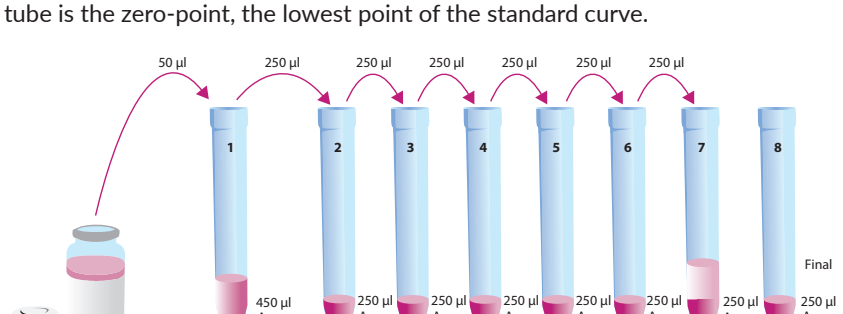


Figure 3. Preparation of glycogen standards - colorimetric

Performing the Assay

NOTE: Use the 96-Well Solid Plate (black) (Item No. 400017) for the fluorometric assay. Use the 96-Well Solid Plate (Colorimetric Assay) (Item No. 400014) for the colorimetric assay.

1. **Standard Wells -** Add 10 µl of standard (tubes 1-8) per well in the designated wells on the plate (see **Sample plate format**, Figure 1, page 11).
2. **Sample Wells -** Add 10 µl 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 µl of the sample and 50 µl of the Glycogen Hydrolysis Buffer (Item No. 700484) to at least two wells.
4. Add 50 µl 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 µl 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 µl 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. *NOTE: Fluorometric detector is used in both the colorimetric and fluorometric assays.*

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

7. Add 150 µl of 'Developer' to all wells being used, including sample, sample background, and standards.
8. Cover and incubate for 15 minutes at 37°C.
9. To read fluorescence: Remove the plate cover and read using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm. To read absorbance (colorimetric): Remove the plate cover and read absorbance at 570nm.

ANALYSIS

Calculations

1. Determine the average fluorescence or absorbance of each standard, sample, and sample background.
2. Subtract the fluorescence or absorbance value of zero standard from itself and all other standards. This is the corrected fluorescence/absorbance.
3. Plot the corrected values (from step 2 above) of each standard as a function of the final concentration of glycogen from Figures 2 and 3. See Figures 4 and 54, on page 18, for typical standard curves.
4. Subtract the average fluorescence/absorbance value of the sample background from the average fluorescence/absorbance of the sample wells to yield the corrected sample measurement (CSM).
5. Calculate the amount of glycogen in of the samples using the equation obtained from the linear regression of the standard curve substituting the CSM for each sample.

$$\text{Glycogen (µg)} = \left[\frac{\text{CSM - (y-intercept)}}{\text{slope}}\right] \text{x sample dilution}$$

Performance Characteristics

Sensitivity:

The LLOQ for the fluorometric assay is 0.003 µg (0.313 µg/ml). The LLOQ for the colorimetric assay is 0.031 µg (3.13 µg/ml).

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%.

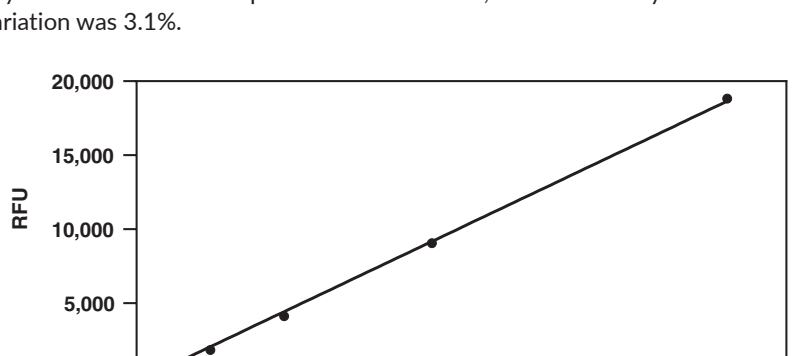


Figure 4. Glycogen standard curve - fluorometric assay

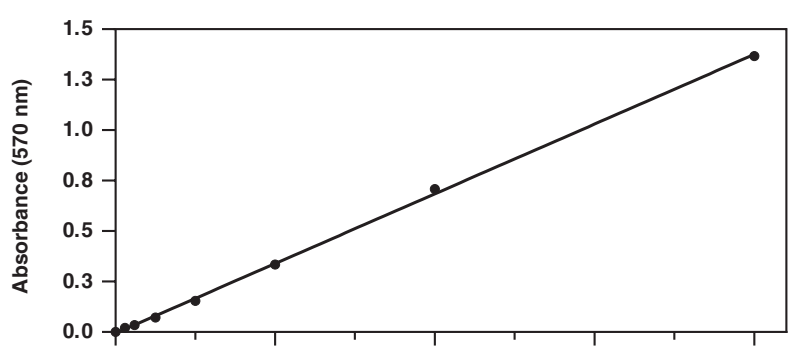


Figure 5. Glycogen standard curve - colorimetric assay

RESOURCES

Interferences

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

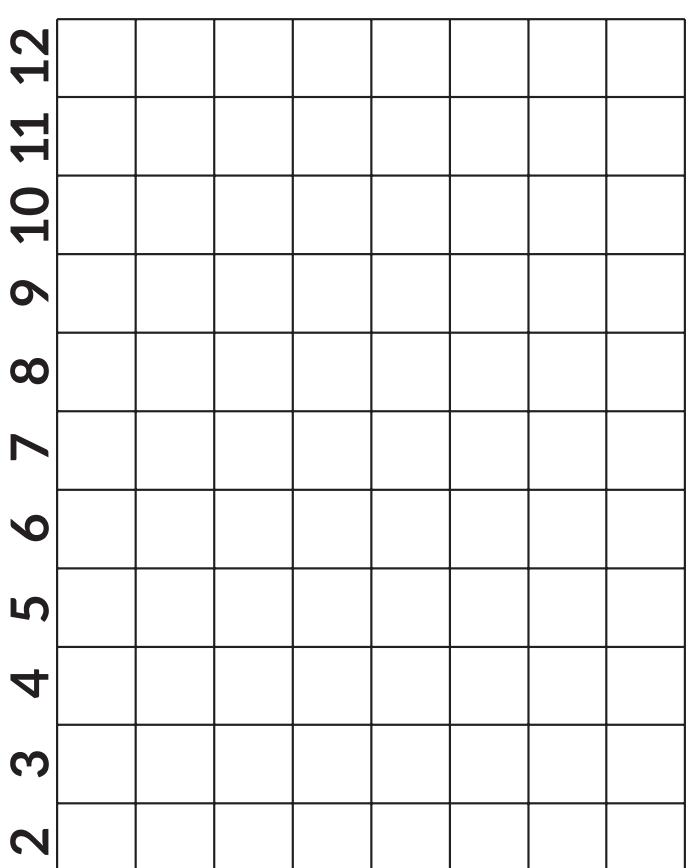
Reagent		Will Interfere (Yes or No)
Buffers	Tris	Yes
	HEPES	No
	Borate	Yes
	MES	No
Detergents	Polysorbate 20 (0.1%)	No
	Polysorbate 20 (1%)	Yes
	Triton X-100 (0.1%)	No
	Triton X-100 (1%)	No
Chelators	EDTA (1 mM)	No
	EGTA (1 mM)	Yes
Protease Inhibitors/ Enzymes	Trypsin (10 µg/ml)	No
	PMSF (200 µM)	No
	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/absorbance	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; *NOTE: Remember to account for the dilution factor when calculating glycogen concentration*
The fluorometer exhibited 'MAX' values for the wells	The *gain* setting is too high	Reduce the *gain* and re-read

References

1. Horton, H.R. Horton, H.R., Moran, L.A., Ochs, R.S. *et al* Neil Patterson Publishers, Englewood Cliffs, NJ, 16-22 (1993).
2. Burton, G.J., Watson, A.L., Hempstock, J., *et al. J. Clin. Endocrinol. Metab.* **87(6)**, 2954-2959 (2002).
3. Hempstock, J., Cindrova-Davies, T., Jauniaux, E., *et al. Reprod. Biol. Endocrinol.* **20(2)**, 58 (2004).
4. Chen, Y.-T. and Burchell, A. criver, C.R., Beaudet, A.L., Sly, W.S. *et al.* McGraw-Hill, New York, 935-965 (1995).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.