ab65620

Glycogen Assay kit
(Colorimetric/Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of glycogen in various samples.

This product is for research use only and is not intended for diagnostic use.
# Table of Contents

**INTRODUCTION**

1. BACKGROUND  2  
2. ASSAY SUMMARY  3

**GENERAL INFORMATION**

3. PRECAUTIONS  4  
4. STORAGE AND STABILITY  4  
5. MATERIALS SUPPLIED  5  
6. MATERIALS REQUIRED, NOT SUPPLIED  5  
7. LIMITATIONS  6  
8. TECHNICAL HINTS  7

**ASSAY PREPARATION**

9. REAGENT PREPARATION  8  
10. STANDARD PREPARATION  9  
11. SAMPLE PREPARATION  11

**ASSAY PROCEDURE and DETECTION**

12. ASSAY PROCEDURE and DETECTION  13

**DATA ANALYSIS**

13. CALCULATIONS  15  
14. TYPICAL DATA  17

**RESOURCES**

15. APPENDIX  18  
16. QUICK ASSAY PROCEDURE  20  
17. FAQs  23  
18. INTERFERENCES  25  
19. NOTES  26
1. **BACKGROUND**

Glycogen Assay Kit (Colorimetric/Fluorometric) (ab65620) is an easy and accurate 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 ($\lambda_{\text{max}} = 570$ nm) and fluorescence (Ex 535/Em 587). This assay can detect glycogen 0.0004 to 2 mg/mL.

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 $\alpha$-1,4 linkage, with branching via $\alpha$-1,6 linkage. Abnormal ability to utilize glycogen is found in diabetes and in several genetic glycogen storage diseases.

. 
2. ASSAY SUMMARY

Sample preparation

Standard curve preparation

Add hydrolysis enzyme mix

Add reaction mix and incubate RT for 30 min

Measure optical density (OD570 nm) or fluorescence (Ex/Em = 535/587 nm)
3. **PRECAUTIONS**

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. **STORAGE AND STABILITY**

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**
5. **MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
<th>Storage Condition (After Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen Hydrolysis Buffer</td>
<td>25 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Glycogen Development Buffer</td>
<td>25 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>OxiRed Probe</td>
<td>200 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Glycogen Hydrolysis Enzyme Mix (Lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Glycogen Development Enzyme Mix (Lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Glycogen Standard (2 mg/mL)</td>
<td>100 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

6. **MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- MilliQ water or other type of double distilled water (ddH₂O)
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader – equipped with filter for OD570 nm or Ex/Em = 535/587 nm (respectively)
- 96 well plate: clear plates for colorimetric assay; black plates (clear bottoms) for fluorometric assay
- Orbital shaker
- Dounce homogenizer (if using tissue)
- Vortex
7. **LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
8. **TECHNICAL HINTS**

- This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

- Keep enzymes and heat labile components and samples on ice during the assay.

- Make sure all buffers and developing solutions are at room temperature before starting the experiment.

- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.

- Avoid foaming or bubbles when mixing or reconstituting components.

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.

- Ensure plates are properly sealed or covered during incubation steps.

- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.

- Make sure you have the appropriate type of plate for the detection method of choice.

- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.
9. **REAGENT PREPARATION**

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Glycogen Hydrolysis Buffer:**

  Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **Glycogen Development Buffer:**

  Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.3 **OxiRed Probe:**

  Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.** Aliquot probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light and moisture. Once the probe is thawed, use with two months.

9.4 **Hydrolysis Enzyme Mix:**

  Reconstitute in 220 µL Hydrolysis Buffer, vortex tubes gently to dissolve. Aliquot enzyme mix so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months.

9.5 **Development Enzyme Mix:**

  Reconstitute in 220 µL Development Buffer, vortex tubes gently to dissolve. Aliquot enzyme mix so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months.

9.6 **Glycogen Standard:**

  Ready to use as supplied. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use. Use within 2 months.
10. **STANDARD PREPARATION**

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 **For the colorimetric assay:**

10.1.1 Prepare 100 µL of 0.2 mg/mL Glycogen standard by adding 10 µL of the Standard to 90 µL of dH2O.

10.1.2 Using 0.2mg/mL standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Volume of Standard (µL)</th>
<th>Assay Buffer (µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End [Glycogen] in well</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>150</td>
<td>50</td>
<td>0 µg/well</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>144</td>
<td>50</td>
<td>0.4 µg/well</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>138</td>
<td>50</td>
<td>0.8 µg/well</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>132</td>
<td>50</td>
<td>1.2 µg/well</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>126</td>
<td>50</td>
<td>1.6 µg/well</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>120</td>
<td>50</td>
<td>2.0 µg/well</td>
</tr>
</tbody>
</table>

Each dilution has enough amount of standard to set up duplicate reading (2 x 50 µL).
10.2 For the flurometric assay:

10.2.1 Prepare 1 mL of 0.02 mg/mL Glycogen Standard by diluting 10 µL of Glycogen Standard to 990 µL of dH2O.

10.2.2 Using 0.02 mg/mL Glycogen standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Volume of Standard (µL)</th>
<th>Assay Buffer (µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End [Glycogen] in well</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>150</td>
<td>50</td>
<td>0 µg/well</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>144</td>
<td>50</td>
<td>0.04 µg/well</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>138</td>
<td>50</td>
<td>0.08 µg/well</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>132</td>
<td>50</td>
<td>0.12 µg/well</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>126</td>
<td>50</td>
<td>0.16 µg/well</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>120</td>
<td>50</td>
<td>0.2 µg/well</td>
</tr>
</tbody>
</table>

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

**NOTE:** If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.
11. **SAMPLE PREPARATION**

**General Sample information:**

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- 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.
- There are a variety of methods for extraction of glycogen from tissues 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 in Section 15.

11.1 **Cell (adherent or suspension) samples:**

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 1 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL dH₂O on ice.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Boil the homogenates for 10 minutes to inactivate enzymes.
11.1.6 Centrifuge the boiled samples at 18,000 x g for 10 min to remove insoluble material.

11.1.7 The supernatant is ready for assay.

11.2 **Tissue samples:**

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg)

11.2.2 Wash tissue in cold PBS

11.2.3 Resuspend tissue in 200 µL of dH₂O.

11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.5 Boil the homogenates for 10 minutes to inactivate enzymes.

11.2.6 Centrifuge the boiled sample at 18,000 x g for 10 minutes.

11.2.7 The supernatant is ready for assay.

11.3 **Plasma, Serum and Urine Samples:**

Plasma, serum and urine sample can be tested directly by adding sample to the microplate wells.

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample (1/2 – 1/5 – 1/10).

**NOTE:** We suggest using different volumes of sample to ensure readings are within the Standard Curve range.
12. ASSAY PROCEDURE and DETECTION

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

12.1 Set up Reaction wells:
- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Hydrolysis Buffer).
- Background wells = 50 µL Hydrolysis Buffer.
- Glucose Background = 1 – 50 µL samples (adjust volume to 50 µL/well with Hydrolysis Buffer).

12.2 Add Hydrolysis Enzyme Mix to standard and sample wells.

<table>
<thead>
<tr>
<th>Component</th>
<th>Colorimetric assay (µL)</th>
<th>Fluorometric assay (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis Enzyme Mix</td>
<td>2 µL</td>
<td>1 µL</td>
</tr>
</tbody>
</table>

12.3 Mix and incubate at room temperature for 30 minutes.

**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.

12.4 Reaction Mix:

Prepare Reaction Mix for each reaction:

<table>
<thead>
<tr>
<th>Component</th>
<th>Reaction Mix Colorimetric (µL)</th>
<th>Reaction Mix Fluorometric (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development Buffer</td>
<td>46</td>
<td>48.7</td>
</tr>
<tr>
<td>Development Enzyme Mix</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>OxiRed Probe</td>
<td>2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a
Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

\[ X \mu L \text{ component } \times (\text{Number samples} + \text{standards} + 1) \]

12.5 Add 50 µL of the Reaction Mix to each well containing Glycogen standard or samples.
12.6 Mix and incubate at room temperature for 30 minutes protected from light.
12.7 Measure output (OD570 nm) or fluorescence (Ex/Em = 535/587 nm) on a microplate reader.
13. **CALCULATIONS**

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of Glycogen.

13.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5 Extrapolate sample readings from the standard curve plotted using the following equation:

\[
Ay = \left( \frac{\text{Corrected absorbance} - (y - \text{intercept})}{\text{Slope}} \right)
\]

13.6 Concentration of samples in the test samples is calculated as:

\[
\text{Glycogen Concentration} = \left( \frac{Ay}{Sv} \right) \times D \quad \mu g/ \mu L
\]

Where:

- Ay = amount of Glycogen (µg) in your sample from the standard curve.
- Sv = sample volume (µL) added to the sample well.
- D = Dilution factor of sample.
13.7 For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

13.8 For spiked samples, the concentration of Glycogen in sample well is calculated as:

\[
\text{Glycogen amount} = \left( \frac{(OD_{\text{sample corrected}})}{(OD_{\text{sample}} + \text{Glycogen std (corrected)} - (OD_{\text{sample corrected}})} \right)
\]

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

Glucose molecular weight: 180.16 g/mol.
14. **TYPICAL DATA**

**TYPICAL STANDARD CURVE** – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

**Figure 1:** Typical Glycogen standard calibration curve using colorimetric reading.

**Figure 2:** Typical Glycogen standard calibration curve using fluorometric reading.
15. APPENDIX

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.

A rapid method useful for small tissue samples is as follows:

- 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 (50 μL) for the colorimetric assay or a proportionally smaller aliquot (10-25 μL) 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°C 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). Re-precipitate 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.
16. **QUICK ASSAY PROCEDURE**

**NOTE:** This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when initially performing the assay.

- Solubilize Hydrolysis Enzyme Mix and Development Enzyme Mix, thaw OxiRed probe and Glycogen Assay Buffer (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL) and samples (50 µL).
- Add Hydrolysis Enzyme Mix (2 µL for colorimetric or 1 µL for Fluorometric) to standard and sample wells.
- Incubate at RT for 30 min.
- Prepare Glycogen Reaction Mix (Number samples + standards + 1)

<table>
<thead>
<tr>
<th>Component</th>
<th>Reaction Mix Colorimetric (µL)</th>
<th>Reaction Mix Fluorometric (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development Buffer</td>
<td>46</td>
<td>48.7</td>
</tr>
<tr>
<td>Development Enzyme Mix</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>OxiRed Probe</td>
<td>2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

- Add 50 µL of the Glycogen Reaction Mix.
- Incubate at RT for 30 mins protected from light.
- Measure plate at OD570 nm for colorimetric or Ex/Em = 535/587 nm for fluorometric.
<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Use of ice-cold buffer</td>
<td>Buffers must be at room temperature</td>
</tr>
<tr>
<td></td>
<td>Plate read at incorrect wavelength</td>
<td>Check the wavelength and filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>Use of inappropriate plate for reader</td>
<td>Colorimetry: Clear plates Fluorescence: Black plates (clear bottom)</td>
</tr>
<tr>
<td>Sample with erratic readings</td>
<td>Samples not deproteinized (if indicated on protocol)</td>
<td>Use PCA precipitation protocol for deproteinization</td>
</tr>
<tr>
<td></td>
<td>Cells/tissue samples not homogenized completely</td>
<td>Use Dounce homogenizer (increase number of strokes); observe for lysis under microscope</td>
</tr>
<tr>
<td></td>
<td>Samples used after multiple free/thaw cycles</td>
<td>Aliquot and freeze samples if needed to use multiple times</td>
</tr>
<tr>
<td></td>
<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use</td>
</tr>
<tr>
<td></td>
<td>Presence of interfering substance in the sample</td>
<td>Check protocol for interfering substances; deproteinize samples</td>
</tr>
<tr>
<td>Lower/Higher readings in samples and Standards</td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
</tr>
<tr>
<td></td>
<td>Allowing reagents to sit for extended times on ice</td>
<td>Always thaw and prepare fresh reaction mix before use</td>
</tr>
<tr>
<td></td>
<td>Incorrect incubation times or temperatures</td>
<td>Verify correct incubation times and temperatures in protocol</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>--------------------------------------------</td>
<td>------------------------------------------------------------</td>
</tr>
<tr>
<td>Standard readings do not follow a</td>
<td>Pipetting errors in standard or reaction</td>
<td>Avoid pipetting small volumes and prepare a master mix</td>
</tr>
<tr>
<td>linear pattern</td>
<td>mix</td>
<td>whenever possible</td>
</tr>
<tr>
<td></td>
<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the tubes</td>
</tr>
<tr>
<td></td>
<td>Standard stock is at incorrect concentration</td>
<td>Always refer to dilutions on protocol</td>
</tr>
<tr>
<td>Unanticipated results</td>
<td>Measured at incorrect wavelength</td>
<td>Check equipment and filter setting</td>
</tr>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>Troubleshoot if it interferes with the kit</td>
</tr>
<tr>
<td></td>
<td>Sample readings above/ below the linear</td>
<td>Concentrate/ Dilute sample so as to be in the linear range</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td></td>
</tr>
</tbody>
</table>
17. FAQs

Where is the glycogen standard from and is it chemically synthesized?
The glycogen standard is from an animal-derived source.

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.

How can mg of glycogen be converted to molar concentration?
Are there other ways to report the data?
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 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 mg of glycogen/mg tissue can be reported.

What are the differences between ab65620 and ab169558?
ab65620 can detect glycogen 0.0004 to 2 mg/ml. This kit can be used in both colorimetric and fluorometric applications. In this assay, glucoamylase hydrolyzes the glycogen to glucose which is then specifically oxidized to produce a product that reacts with OxiRed probe to generate color (λmax= 570 nm) and fluorescence (Ex 535/Em 587).
Since the fluorometric version is very sensitive, this improved kit uses glycogen extraction and removes all enzyme activity by boiling to reduce glycogenolysis and the samples can be stored after this step to be assayed later.
ab169558: can detect less than 4 µg/ml of glycogen in samples. This kit is colorimetric only. This assay is suitable for measuring glycogen levels in samples that contain reducing substances, which may interfere with the oxidase based assays. In this assay, glycogen is
hydrolyzed into glucose, which is oxidized to form an intermediate that reduces a colorless probe to a colored product with strong absorbance at 450 nm. This is a faster protocol without any glycogen extraction but not as sensitive as the fluorometric version of ab65620.
18. **INTERFERENCES**

These chemicals or biological will cause interferences in this assay causing compromised results or complete failure.

- Urine disphosphate glucose (UDP) - whether UDP glucose will interfere or not depends on the amount of UDP glucose compared to glycogen in the sample. A background control can be run in parallel to measure any interference from endogenous glucose/UDP glucose. The oxidation of glucose in the assay leads eventually to color development. It is possible that UDP glucose competes at this step but we have not tested this.
19. **NOTES**