ab65620
Glycogen Assay Kit
(Colorimetric/Fluorometric)

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.
1. Overview

Glycogen Assay Kit (Colorimetric/ Fluorometric) (ab65620) is an easy and accurate assay to measure glycogen levels in biological samples such as cell and tissue extract, as well as fluids. In the assay, glucoamylase, an alpha-glucosidase digestive enzyme, hydrolyzes the glycogen to glucose which is then specifically oxidized to produce a product that reacts with OxiRed probe to generate color (ODmax = 570 nm) or fluorescence (Ex/Em = 535/587 nm). This assay can detect glycogen 0.4 μg/mL – 2 mg/mL.

Reducing substances will interfere with this assay. If your sample is likely to contain reducing substances, we recommend using Glycogen Assay Kit II (ab169558).

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.
2. Protocol Summary

Standard curve preparation

↓

Sample preparation

↓

Add hydrolysis enzyme mix and incubate RT for 30 minutes

↓

Add reaction mix and incubate RT for 30 minutes

↓

Measure optimal 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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

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 the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

△ Note: Reconstituted components are stable for 2 months.
5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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.

6. Materials Supplied

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage condition (before prep)</th>
<th>Storage condition (after prep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen Development Buffer</td>
<td>25 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Glycogen Hydrolysis Buffer</td>
<td>25 mL</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 Hydrolysis 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>
<tr>
<td>OxiRed Probe</td>
<td>200 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
</tbody>
</table>
7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at OD 570 nm (colorimetric) or fluorescence at Ex/Em = 535/587 nm (fluorometric)
- MilliQ water or other type of double distilled water (ddH₂O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom (for colorimetric assay) / 96 well plate with clear flat bottom, preferably black (for fluorometric assay)
- Dounce homogenizer (if using tissue)
- Protein quantification method – we recommend BCA protein assay reducing agent compatible (microplate) (ab207003) or BCA protein assay kit for low concentrations (ab207002)

Optional – for tissues with large volume of sample (>100 mg):

- Potassium hydroxide (KOH), 30%
- Ethanol, 95%
- Hydrochloric acid (HCl), 5N
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.

- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.

- Avoid foaming or bubbles when mixing or reconstituting components.

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

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

- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.

- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.

- Make sure all necessary equipment is switched on and set at the appropriate temperature.
9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Glycogen Development Buffer:
Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 Glycogen Hydrolysis Buffer:
Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.3 Glycogen Development Enzyme Mix (lyophilized):
Reconstitute in 220 µL Glycogen Development Buffer. Gently vortex tubes to dissolve. Keep on ice during the assay. Aliquot developer so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within two months.

9.4 Glycogen Hydrolysis Enzyme Mix (lyophilized):
Reconstitute in 220 µL Glycogen Hydrolysis Buffer. Gently vortex tubes to dissolve. Keep on ice during the assay. Aliquot developer so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within two months.

9.5 Glycogen Standard (2 mg/mL):
Ready to use as supplied. Keep on ice while in use. Aliquot standard so that you have enough to perform the desired number of assays. Store at -20°C.

9.6 OxiRed Probe:
Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 min 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 a 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. Once the probe is thawed, use within two months.
10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 For colorimetric assay:

10.1.1 Prepare 100 µL of 0.2 mg/mL standard by diluting 10 µL of the provided standard (2 mg/mL solution) with 90 µL of ddH$_2$O.

10.1.2 Using 0.2 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>Glycogen 0.2 mg/mL Standard (µL)</th>
<th>Hydrolysis Buffer (µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End amount Glycogen in well (µg/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>150</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>144</td>
<td>50</td>
<td>0.4</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>138</td>
<td>50</td>
<td>0.8</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>132</td>
<td>50</td>
<td>1.2</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>126</td>
<td>50</td>
<td>1.6</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>120</td>
<td>50</td>
<td>2</td>
</tr>
</tbody>
</table>

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).
10.2 For fluorometric Assay:

10.2.1 Prepare 1 mL of 0.02 mg/mL Glycogen standard by diluting 10 µL of the provided standard (2 mg/mL solution) with 990 µL of ddH₂O.

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>Glycogen 0.02mg/mL Standard (µL)</th>
<th>Hydrolysis Buffer (µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End amount Glycogen in well (µg/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>150</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>144</td>
<td>50</td>
<td>0.04</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>138</td>
<td>50</td>
<td>0.08</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>132</td>
<td>50</td>
<td>0.12</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>126</td>
<td>50</td>
<td>0.16</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>120</td>
<td>50</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).
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. Alternatively, snap freeze your samples in liquid nitrogen upon extraction and store them 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.
- In some tissues (such as neural tissue), rapid rates of anaerobic metabolism continue to happen even after death, causing rapid decline in glucose levels within few seconds. Utilization of glycogen storage follows and a large decrease in glycogen content can be observed within less than a minute. For an accurate measurement of glycogen in such tissue types, metabolic activity should be quenched by freeze clamping or immediate submersion of tissue in liquid nitrogen, followed by grinding in liquid nitrogen and storage at -80°C.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 1 x 10⁶ cells).
11.1.2 Wash cells with cold PBS.
11.1.3 Resuspend cells in 200 µL of ddH₂O 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 in the sample.
11.1.6 Centrifuge boiled samples 10 minutes at 4°C at 18,000 x g in a cold microcentrifuge to remove any insoluble material.
11.1.7 Collect supernatant and transfer to a new tube.
11.1.8 Optional: Determine sample concentration (using a standard method such as BCA) by extracting a portion of your sample.
11.2 Tissue – small amount of sample (< 100 mg):
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 ddH₂O (approx. 50 volumes) on ice.
11.2.4 Homogenize tissue with a Dounce homogenizer or pestle sitting on ice, with 10 – 15 passes.
11.2.5 Boil the homogenates for 10 minutes to inactivate enzymes in the sample.
△ Note: If sample will not be assayed immediately, store at -20°C until used. Do not store for more than 1 month.
11.2.6 Centrifuge boiled samples 10 minutes at 4°C at 18,000 x g in a cold microcentrifuge to remove any insoluble material.
11.2.7 Collect supernatant and transfer to a new tube.
11.2.8 Optional: Determine sample concentration (using a standard method such as BCA) by extracting a portion of your sample.

11.3 Tissue – large amount of sample (> 100 mg):
This procedure should be used when sample is sufficient large (from few hundred milligrams to grams).
11.3.1 Harvest the amount of tissue necessary for each assay.
11.3.2 Wash tissue in cold PBS.
11.3.3 Resuspend tissue in 30% KOH to a final content of 30% - 50%.
11.3.4 Heat sample to 100°C for 2 hours.
11.3.5 Cool sample down and add 2 volumes of 95% Ethanol. This will precipitate the crude glycogen.
11.3.6 Centrifuge samples 10 minutes at 4°C at 18,000 x g in a cold microcentrifuge to remove any insoluble material. Discard supernatant.
11.3.7 Dissolve or resuspend the precipitate in a minimal amount of ddH₂O (enough to dissolve pellet) and acidify sample to pH 3 with HCl 5N, drop by drop.
11.3.8 Re-precipitate with 1 volume of 95% Ethanol.
11.3.9 Repeat procedure from Step 11.3.4 – Step 11.3.8 two more times.
11.3.10 Wash precipitate in 95% Ethanol and dry.
△ Note: This procedure removes the vast majority of the glucose background with minimal effect on the glycogen. The dried material
can be weighed and dissolved in Glycogen Hydrolysis Buffer for analysis.

11.3.11 Optional: Determine sample concentration (using a standard method such as BCA) by extracting a portion of your sample.

11.4 Plasma, Serum and Urine samples:
Plasma, serum and urine samples can be tested directly by adding sample to the microplate assay wells.

Use heparin when collecting plasma or serum.
To find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample.

\textbf{Note:} Endogenous compounds in the sample 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).
12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.
- Samples from tissues with high glycogen content (liver, muscle) generally only require 5 – 25 µL sample to give a clearly measurable colorimetric signal. Samples from tissues with low glycogen content generally require 50 µL sample in the colorimetric assay, or should be tested in the more sensitive fluorometric assay (10 – 25 µL).

△ Note: Glucose present in cell or tissue extracts can generate background in this assay. If you suspect your samples contain glucose, set up Sample Background Controls.

12.1 Set up Reaction wells:
- Standard wells = 50 µL standard dilutions.
- Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Hydrolysis Buffer).
- Sample Background Control wells = 2 – 50 µL samples (adjust volume to 50µL/well with Hydrolysis Buffer).

12.2 COLORIMETRIC ASSAY procedure:
12.2.1 Add 2 µL of Hydrolysis Enzyme Mix to standard and sample wells. Do not add Hydrolysis mix to background controls.
12.2.2 Mix well and incubate at room temperature for 30 minutes.
12.2.3 Prepare 50 µL of Reaction Mix for each reaction. Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction mix to ensure consistency.
<table>
<thead>
<tr>
<th>Component</th>
<th>Colorimetric Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development Buffer</td>
<td>46</td>
</tr>
<tr>
<td>Development Enzyme Mix</td>
<td>2</td>
</tr>
<tr>
<td>OxiRed Probe</td>
<td>2</td>
</tr>
</tbody>
</table>

12.2.4 Add 50 µL of Reaction Mix into each standard, sample and sample background wells.
12.2.5 Mix and incubate at room temperature for 30 min protected from light.
12.2.6 Measure output immediately after incubation on a microplate reader at OD 570 nm.

12.3 FLUOROMETRIC ASSAY procedure:
12.3.1 Add 1 µL of Hydrolysis Enzyme Mix to standard and sample wells. Do not add Hydrolysis mix to background controls.
12.3.2 Mix well and incubate at room temperature for 30 minutes.
12.3.3 Prepare 50 µL of Reaction Mix for each reaction. Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction mix to ensure consistency.

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

12.3.4 Add 50 µL of Reaction Mix into each standard, sample and sample background wells.
12.3.5 Mix and incubate at room temperature for 30 min protected from light.
12.3.6 Measure output immediately after incubation on a microplate reader at Ex/Em = 535/587 nm.
13. Calculations

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

13.1 Subtract the sample background control from sample reading.
13.2 Average the duplicate reading for each standard and sample.
13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of Glycogen.
13.5 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).
13.6 Concentration of Glycogen (µg/µL) in the test samples is calculated as:

\[
\text{Glycogen concentration} = \frac{T_s}{S_v} \times D
\]

Where:
Ts = amount of glycogen in the sample well calculated from standard curve (µg).
Sv = sample volume added in the sample wells (µL).
D = sample dilution factor.

Glycogen molecular size = ~60,000 glucose molecules (10^6 – 10^7 daltons)
Glycogen molecular weight = 180.16 g/mol
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:

\[
\text{Glycogen} = \left(\frac{\text{ODs cor}}{\text{ODs} + \text{Ts cor} - \text{ODs cor}}\right) \times \text{Glycogen spike (µg)}
\]

Where:
ODs cor = OD sample corrected
ODs = OD sample
Ts cor = amount of glycogen from standard curve corrected
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 fluorometric reading.
Figure 2. Quantification of glycogen in various mouse tissues and fluids using fluorometric assay. Sample was diluted 1:54 in assay buffer. Background readings have been subtracted.
Figure 3. Quantitation of glycogen in MBA-MB—231 cells (human breast adenocarcinoma cell line). 10⁶ cells were prepared following protocol instructions, and several dilutions were measured using fluorometric detection. Background readings have been subtracted.
15. Quick Assay Procedure

△ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Solubilize Hydrolysis enzyme mix, Development enzyme mix and Glycogen standard (aliquot if necessary), thaw OxiRed probe and buffers; get equipment ready.
- Prepare Glycogen standard dilution for your desired detection method: colorimetric [0.4 – 2 µg/well] or fluorometric [0.04 – 0.2 µg/well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL), samples (50 µL) and background sample control wells (50 µL).
- Add Hydrolysis Enzyme Mix (2 µL for colorimetric assay; 1 µL for fluorometric assay) to standard and sample wells only.
- Incubate plate at RT for 30 minutes.
- Prepare a master mix for Glycogen Reaction Mix:

<table>
<thead>
<tr>
<th>Component</th>
<th>Colorimetric Reaction Mix (µL)</th>
<th>Fluorometric Reaction Mix (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 Glycogen Reaction Mix to all wells.
- Incubate plate at RT for 30 minutes protected from light.
- Measure plate at OD 570 nm for colorimetric assay or at Ex/Em= 535/587 nm for fluorometric assay.
### 16. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay not working</strong></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><strong>Sample with erratic readings</strong></td>
<td>Samples not deproteinized (if indicated on protocol)</td>
<td>Use provided protocol for deproteinization</td>
</tr>
<tr>
<td></td>
<td>Cells/tissue samples not homogenized completely</td>
<td>Use Dounce homogenizer, increase number of strokes</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><strong>Lower/higher readings in samples and standards</strong></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>Reason</td>
<td>Solution</td>
</tr>
<tr>
<td>---------</td>
<td>--------</td>
<td>----------</td>
</tr>
<tr>
<td>Standard readings do not follow a linear pattern</td>
<td>Pipetting errors in standard or reaction mix</td>
<td>Avoid pipetting small volumes (&lt; 5 µL) and prepare a master mix 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 described in the 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 range</td>
<td>Concentrate/ Dilute sample so it is within the linear range</td>
</tr>
</tbody>
</table>
17. Interferences

These chemical or biological materials will cause interferences in this assay causing compromised results or complete failure:

- Urine diphosphate 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. Oxidation of glucose in the assay leads eventually to color development. It is possible that UDP glucose competes in this late step but it has not been tested.

18. FAQs

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

Q. Can you recommend a good positive control sample tissue for glycogen?
A. Liver is the main site for glycogen storage. Skeletal muscle generally contains high amount of glycogen too (although much less than liver).

Q. Is there any glucose in the glycogen standard?
A. There should not be any glucose in the glycogen standard. Glucose in the samples can generate background in this assay.

Q. How can mg of glycogen be converted to molar concentration? Are there other ways to report the data?
A. mg of glycogen can be converted to mmol 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.
Q. What are the differences between ab65620 and ab169558?
A. 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 ($\lambda_{max}= 570$ 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.
19. Notes
Technical Support

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