Instructions for use:

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

This product is for research use only and is not intended for diagnostic use.
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1. BACKGROUND

Glucose Assay Kit (Colorimetric/Fluorometric) (ab65333) provides direct measurement of glucose in various biological samples such as, cell lysates, biological fluids and growth medium). The assay is also suitable for monitoring glucose level during fermentation and glucose feeding in protein expression processes. The glucose enzyme mix specifically oxidizes glucose to generate a product which reacts with a dye to generate color ($\lambda = 570$ nm) or fluorescence ($\text{Ex}/\text{Em} = 535/587$ nm). The generated color and fluorescence is proportional to the amount of glucose present in the sample.

The method is rapid, simple, sensitive, and suitable for high throughput. The kit detects 1-10000 µM glucose in samples.

Principle:

\[
\text{Glucose} + \text{O}_2 \xrightarrow{\text{Glucose Oxidase}} \text{Gluconolactone} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + \text{Probe} \xrightarrow{\text{HRP}} \text{OD 570nm or 535/587nm}
\]

Glucose ($\text{C}_6\text{H}_{12}\text{O}_6$; MW: 180.16 g/mol) is a very important fuel source to generate universal energy molecules such as ATP. Glucose level is a key diagnostic parameter for many metabolic disorders. Measurement of glucose can be very important in both research and drug discovery processes.
2. ASSAY SUMMARY

- Standard curve preparation

- Sample preparation *

- Prepare and add reaction mix and incubate for 30 minutes 37°C in the dark

- Measure absorbance (OD570 nm) or Fluorescence (Ex/Em = 535/587 nm)

*Sample require deproteinization
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 handle 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. **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>Amount</th>
<th>Storage Condition (Before Preparation)</th>
<th>Storage Condition (After Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Assay Buffer</td>
<td>25 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Glucose Probe (in DMSO)</td>
<td>200 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Glucose Enzyme Mix (Lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>100 nmol/µL Glucose Standard</td>
<td>100 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 (ddH$_2$O)
- 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)
- Deproteinizing Sample Preparation Kit – TCA (ab204708): for deproteinization step in cell or tissue samples
- 10 kD Spin Columns (ab93349): for deproteinization step in fluid samples
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 you have the right type of plate for your detection method of choice.

- 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. Glucose Assay Buffer:
Ready to use as supplied. Equilibrate to room temperature prior to use. Store at -20°C.

9.2. Glucose Probe:
Ready to use as supplied. 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 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.

9.3. Glucose Enzyme Mix:
Reconstitute in 220 µL Glucose Assay Buffer. Keep on ice during the assay. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C. Use within two months.

9.4. Glucose Standard (100 nmol/µL):
Ready to use as supplied. Equilibrate to room temperature prior to use. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C.
ASSAY PREPARATION

10. STANDARD PREPARATION

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

10.1. **For colorimetric assay:**

10.1.1. Prepare a 1 mL of 1 nmol/µL Glucose standard by diluting 10 µL of the Glucose Standard (section 9.4) in 990 µL of Glucose Assay Buffer.

10.1.2. Using 1 nmol/µL Glucose 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 Glucose standard (µL)</th>
<th>Assay Buffer (µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End [Glucose] in well (nmol/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>2</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>138</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>132</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>126</td>
<td>50</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>120</td>
<td>50</td>
<td>10</td>
</tr>
</tbody>
</table>

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

10.2.1. Prepare a 1 nmol/µL Glucose standard by diluting 5 µL of the Glucose Standard (section 9.4) in 495 µL of Glucose Assay Buffer.

10.2.2. Prepare a 0.1 nmol/µL Glucose standard by diluting 100 µL of 1 nmol/µL Glucose Standard with 900 µL of Glucose Assay Buffer.

10.2.3. Using 0.1 nmol/µL Glucose 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 Glucose standard (µL)</th>
<th>Assay Buffer (µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End [Glucose] in well (nmol/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>0.2</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>138</td>
<td>50</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>132</td>
<td>50</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>126</td>
<td>50</td>
<td>0.8</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>120</td>
<td>50</td>
<td>1</td>
</tr>
</tbody>
</table>

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

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 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.

11.1. Cell (adherent or suspension) samples:

11.1.1. Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2. Wash cells with cold PBS.

11.1.3. Resuspend cells in 100 µL of Assay Buffer.

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

11.1.5. Centrifuge 2 minutes at 4°C at top speed in a cold microcentrifuge to remove any insoluble material.

11.1.6. Collect supernatant and transfer to a clean tube.

11.1.7. Keep on ice.

11.1.8. Cell samples may contain enzymes that can interfere with the assay. Remove these enzymes from sample by using Deproteinizing Sample Preparation Kit – TCA (ab204708). Alternatively, you can perform a PCA/KOH deproteinization step following the protocol described in section 11.4.
11.2. **Tissue samples:**

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

11.2.2. Wash tissue in cold PBS.

11.2.3. Resuspend tissue in 100 µL of Assay Buffer.

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

11.2.5. Centrifuge sample for 2 – 5 minutes at top speed at 4°C in a cold microcentrifuge to remove any insoluble material.

11.2.6. Collect supernatant and transfer to a clean tube.

11.2.7. Keep on ice.

11.2.8. Tissue samples may contain enzymes that can interfere with the assay. Remove these enzymes from sample by using Deproteinizing Sample Preparation Kit – TCA (ab204708). Alternatively, you can perform a PCA/KOH deproteinization step following the protocol described in section 11.4.

11.3. **Plasma, serum, urine and other biological fluids:**

**NOTE:** Limit serum sample volume to 0.5 – 2 µL/ well. Adjust the volume to 50 µL with Glucose Assay Buffer.

Biological fluid samples generally contain high amount of proteins which can interfere with the assay. Remove these enzymes from sample by using a 10kD Spin column (ab93349). Add sample to the spin column, centrifuge at 10,000 x g for 10 minutes at 4°C and collect the filtrate.

11.4. **Alternative deproteinization protocol:**

For this step you will need additional reagents:

- Perchloric acid (PCA) 4M, ice cold
- Potassium hydroxide (KOH), 2M

Prepare samples as specified in protocol. You should have a clear protein sample after homogenization and centrifugation. Keep your samples on ice.
11.4.1. Add PCA to a final concentration of 1M in the homogenate solution and vortex briefly to mix well. **NOTE:** **high protein concentration samples might need more PCA.**

11.4.2. Incubate on ice for 5 minutes.

11.4.3. Centrifuge samples at 13,000 \( \times \) \( g \) for 2 minutes at 4°C in a cold centrifuge and transfer supernatant to a fresh tube. Measure volume of supernatant.

11.4.4. Precipitate excess PCA by adding ice-cold 2M KOH that equals 34% of the supernatant to your sample (for instance, 34 µL of 2 M KOH to 100 µL sample) and vortexing briefly. This will neutralize the sample and precipitate excess PCA.

11.4.5. After neutralization, it is very important that pH equals 6.5 – 8 (use pH paper to test 1 µL of sample). If necessary, adjust pH with 0.1 M KOH or PCA.

11.4.6. Centrifuge at 13,000 \( \times \) \( g \) for 15 minutes at 4°C and collect supernatant.

11.4.7. Samples are now deproteinized, neutralized and PCA has been removed. The samples are now ready to use in the assay.
Sample Recovery
The deproteinized samples will be diluted from the original concentration.
To calculate the dilution factor of your final sample, simply apply the following formula:

\[
\% \text{ original concentration} = \frac{\text{Initial sample volume}}{\text{Initial sample volume} + \text{Vol PCA} + \text{Vol KOH}} \times 100
\]

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

*Endogenous compounds in the sample may interfere with the reaction. To ensure accurate determination of Glucose in the test samples, we recommend spiking samples with a known amount of Standard (4 nmol).*
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.

**NOTE:** High concentration of sucrose present in cell or tissue extracts can generate background in this assay. If you suspect your samples contain sucrose, 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 Glucose Assay Buffer).

Sample Background control wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Glucose Assay Buffer).

12.2. Glucose Reaction Mix (COLORIMETRIC ASSAY):

12.2.1. 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. We recommend the following calculation:

\[ X \times \text{µL component} \times (\text{Number reactions} + 1) \]

<table>
<thead>
<tr>
<th>Component</th>
<th>Reaction Mix (µL)</th>
<th>Background Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Assay Buffer</td>
<td>46</td>
<td>48</td>
</tr>
<tr>
<td>Glucose Probe</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Glucose Enzyme Mix</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

12.2.2. Add 50 µL of Reaction Mix into each standard and samples wells.

12.2.3. Add 50 µL Background Reaction Mix into the Sample Background control wells.
ASSAY PROCEDURE

12.2.4. Mix and incubate for 30 minutes at 37°C protected from light.

12.2.5. Measure output on a microplate reader at OD 570 nm.

12.3. **Glucose Reaction Mix (FLUOROMETRIC ASSAY):**

12.3.1. 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. We recommend the following calculation: $X \mu L \text{ component } x (\text{Number reactions } + 1)$.

<table>
<thead>
<tr>
<th>Component</th>
<th>Reaction Mix (µL)</th>
<th>Background Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Assay Buffer</td>
<td>47.6</td>
<td>49.6</td>
</tr>
<tr>
<td>Glucose Probe*</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Glucose Enzyme Mix</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

*NOTE: For fluorometric readings, using 0.4µL/well of the Glucose probe decreases the background readings, therefore increasing detection sensitivity.*

12.3.2. Add 50 µL of Reaction Mix into each standard and sample wells.

12.3.3. Add 50 µL Background Reaction Mix into the sample background control wells.

12.3.4. Mix and incubate for 30 minutes at 37°C protected from light.

12.3.5. Measure output 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. Average the duplicate reading for each standard and sample.

13.2. If the sample background control is significant, then subtract the sample background control from sample reading.

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 Glucose.

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 glucose in the test samples is calculated as:

\[
Glucose\ concentration = \left( \frac{S_a}{S_v} \right) \times D
\]

Where:

- \(S_a\) = amount of Glucose in the sample well calculated from the standard curve (nmol).
- \(S_v\) = volume of sample added into the reaction well (\(\mu\)L).
- \(D\) = sample dilution factor.

Glucose molecular weight: 180.2 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 Glucose in sample well is calculated as:

\[
\text{Glucose} = \left( \frac{(ODs \text{ cor})}{(ODs + Sa \text{ cor})} \right) \times \text{Glucose spike (nmol)}
\]

Where:
- ODs cor = OD sample corrected
- ODs = OD sample
- Sa cor = Glucose amount from standard
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 Glucose Standard calibration Curve following colorimetric protocol.

**Figure 2:** Typical Glucose Standard calibration Curve following fluorometric protocol.
Figure 3: Quantitation of Glucose in human urine & serum. Urine and serum samples were deproteinized using a 10kD Spin column (ab93349) (10,000xg, 10 min, 4°C). Urine filtrate (20 µL) and serum filtrate (1 µL) were spiked with a known amount of glucose as internal standard (4 nmol).

Figure 4: Glucose measured in cell lysates (nmol) per million cells. Initial concentration of samples was 2 x 107 cells/mL. Samples were diluted 1.5-13.5 fold and measured colorimetrically.
Figure 5: Glucose measured in human biological fluids showing quantity (µmol) per mL of tested sample. Samples were diluted 13.5 fold and measured colorimetrically.
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 Glucose Standard, thaw Glucose Probe and prepare enzyme mix (aliquot if necessary); get equipment ready.
- Prepare Glucose standard dilution for your desired detection method: colorimetric [2 – 10 nmol/well] or fluorometric [0.2 – 1 nmol/well].
- Prepare samples (including deproteinization step) 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).
- Prepare a master mix for Glucose Reaction Mix and (if appropriate) a master mix for Background Reaction Mix:

<table>
<thead>
<tr>
<th>Component</th>
<th>Colorimetric / Background Reaction Mix (µL)</th>
<th>Fluorometric / Background Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Assay Buffer</td>
<td>46 / 48</td>
<td>47.6 / 49.6</td>
</tr>
<tr>
<td>Glucose Probe</td>
<td>2 / 2</td>
<td>0.4 / 0.4</td>
</tr>
<tr>
<td>Glucose Enzyme Mix</td>
<td>2 / 0</td>
<td>2 / 0</td>
</tr>
</tbody>
</table>

- Add 50 µL Reaction Mix to standard and sample wells.
- Add 50 µL Background Reaction Mix to Sample Background control wells.
- Incubate plate at 37°C 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>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 a different 96-well plate</td>
<td>Colorimeters: Clear plates Fluorometric: black wells/clear bottom plate</td>
</tr>
<tr>
<td>Sample with erratic readings</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); 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 linear pattern</td>
<td>Pipetting errors in standard or reaction mix</td>
<td>Avoid pipetting small volumes 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 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 range</td>
<td>Concentrate/ Dilute sample so as to be in the linear range</td>
</tr>
</tbody>
</table>
17. INTERFERENCES
These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- Sucrose
- Peroxide
- Reducing agents – reducing agents will interfere with this assay. If sample contains reducing substances, we recommend using Glucose Detection Kit II (ab102517).

18. FAQs
Will the phenol red in the media affect the assay readout?
Very low amounts of media are used for each sample. This will generate a very low background at the best. Please use only media as a background control and subtract this reading from all sample readings to accommodate for the phenol red.

Why does the final color development start as pink, then goes to brown then disappears?
This is a very common phenomenon observed with use of the OxiRed probe (contained in the Glucose Probe) and is caused due to excessive analyte concentration in the samples. Therefore, if your glucose samples are too concentrated, you may see this. Please dilute your samples with the assay buffer before reanalyzing.

Will this kit detect glucose in purified polysaccharide samples?
This kit has not been tested in purified polysaccharide samples. The first step of the reaction is specific for glucose so it should be possible. However, background might be an issue so we recommend doing a background control check.
19. NOTES
UK, EU and ROW
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