Version 3h Last updated 25 March 2024

ab219924 Glucose Oxidase Activity Assay Kit (Colorimetric)

For the rapid, sensitive and accurate measurement of Glucose Oxidase activity in a variety of samples.

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

Table of Contents

1. Overview

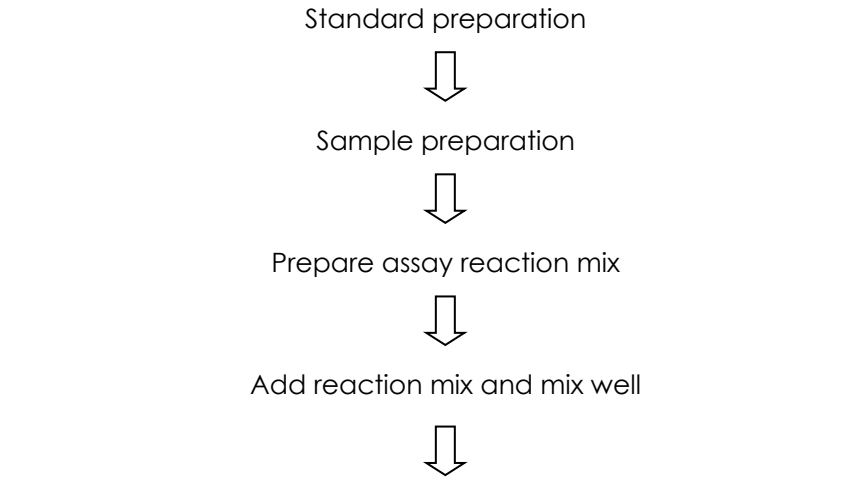
Glucose Oxidase Activity Assay Kit (Fluorometric) (ab219924) provides a simple method to measure glucose oxidase (GOx) activity in cell and tissue extracts, as well as physiological solutions. The assay uses our AbRed Indicator to monitor glucose oxidase activity, leading to an increase in absorbance that can be easily detected at OD 575 nm.

The assay can be performed in a convenient 96-well or 384-well plate format and is easily adapted to automation without a separation or wash step.

Glucose oxidase (notatin, GOx, EC 1.1.3.4) is a dimeric enzyme that catalyzes oxidation of beta-D-glucose into hydrogen peroxide and D-glucono-1,5-lactone, which is hydrolyzed to gluconic acid. This enzyme is produced by certain species of fungi and insects and displays antibacterial activity when oxygen and glucose are present.

Glucose oxidase is widely used for the determination of glucose in body fluids and in removing residual glucose and oxygen from beverages, food and other agricultural products. Furthermore, Glucose oxidase is commonly used in biosensors to detect glucose.

2. Protocol Summary



** For kinetic mode detection, incubation time given in this summary is for guidance only*

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 desiccated 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.

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

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer	50 mL	-20°C	-20°C / 4°C
DMSO	200 µL	-20°C	-20°C
AbRed Indicator	1 vial	-20°C	-20°C
Glucose	1 vial	-20°C	-20°C
Glucose Oxidase (100 U)	1 vial	-20°C	-20°C
Horseradish peroxidase (HRP)	1 vial	-20°C	-20°C

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
- PBS
- 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
- Cell scraper (for adherent cells)
- (Optional)Mammalian Cell Lysis Buffer 5X (ab179835)

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 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.
- 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 **Assay Buffer (50 mL):**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C or 4°C.

9.2 **DMSO (200 µL):**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 min to thaw the DMSO solution before use.

Δ 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. Repeat this step every time probe is needed.

Store at -20°C protected from light.

9.3 **AbRed Indicator (lyophilized):**

Dissolve AbRed Indicator in 100 µL DMSO and mix thoroughly by pipetting up and down. Label this component **250X AbRed Stock Solution**. Keep on ice during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze-thaw cycles. Use within two months.

9.4 **Glucose (lyophilized):**

Dissolve Glucose in 5 mL Assay Buffer and mix thoroughly by pipetting up and down. Label this component **10X Glucose Stock Solution**. Keep on ice during the assay. Aliquot glucosee so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze-thaw cycles. Use within two months.

9.5 **Glucose Oxidase (lyophilized, 100 Units):**

Dissolve Glucose oxidase in 1 mL Assay Buffer and mix thoroughly by pipetting up and down to make a 100 U/mL Glucose Oxidase Stock Solution. Keep on ice during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze-thaw cycles. Use within two months.

9.6 **Horseradish Peroxidase:**

Dissolve Horseradish Peroxidase (HRP) in 1 mL of Assay Buffer and mix thoroughly by pipetting up and down. Label this component **50X HRP Stock Solution**. Keep on ice during the assay. Aliquot HRP solution so that you have enough volume to perform the desired number of assays. Store at - 20°C. 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 Prepare a glucose oxidase standard by diluting 2µL of the 100U/mL glucose oxidase stock solution (from Step 9.5) into 198µL of Assay Buffer to have 1U/mL glucose oxidase standard solution.
Using the 1U/mL Glucose Oxidase standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Sample to dilute	Volume standard in well (µL)	Assay Buffer (µL)	End activity GOx in well (mU/mL)
1	1 U/mL	4	396	10
2	Std #1	200	200	5
3	Std #2	200	200	2.5
4	Std #3	200	200	1.25
5	Std #4	200	200	0.625
6	Std #5	200	200	0.313
7	Std #6	200	200	0.156
8 (blank)	0	0	200	0

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.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, 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.
- Samples prepared by other protocols can be used as well for this assay. Do not use RIPA buffer as it will interfere with the assay. If you have your samples ready, please skip this section and proceed to Assay Procedure section.

Δ Note: AbRed Indicator is unstable in the presence of thiols such as DTT and β-mercaptoethanol. Presence of thiols at a concentration > 10 µM will significantly decrease the assay dynamic range.

11.1 Cell lysates:

Δ Note: For ease of use, mammalian adherent or suspension cells lysates can be easily prepared using Mammalian Cell Lysis Buffer 5X (ab179835). Follow product protocol and proceed to Section 12.

11.1.1 Harvest the number of cells necessary for each assay (initial recommendation: 2-5 x 10^5 cells).
11.1.2 Wash cells with cold PBS.
11.1.3 Resuspend or scrape cells in 100 µL of cold PBS.
11.1.4 Homogenize cells quickly by pipetting up and down a few times.
11.1.5 Centrifuge 5 minutes at 4°C at 13,000 x*g* in a cold microcentrifuge to remove any insoluble material.
11.1.6 Collect supernatant and transfer to a new tube.
11.1.7 Keep on ice.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.
- Prepare all reagents and samples as directed in the previous sections.
- The protocol describe in this section is for 1 x 96-well plate. To perform the assay in a 384-wp, scale down volumes by half.

Δ Note: AbRed Indicator is unstable in the presence of thiols such as DTT and β-mercaptoethanol. Presence of thiols at a concentration > 10 µM will significantly decrease the assay dynamic range.

AbRed Indicator is also unstable at high pH (pH >8.5). Reaction should be performed at pH 7-8. We recommend using the provided Assay Buffer (pH 7.4).

12.1 Set up reaction wells:

- Blank control = 50 µL Assay Buffer.
- Standard wells = 50 µL standard dilutions.
- Sample wells = 1-50 µL samples (adjust volume to 50 µL/well with Assay Buffer).

12.2 Glucose Oxidase assay reaction:

12.2.1 Prepare Glucose Oxidase reaction mix as described in the table below. The volume given in the table is enough for 1 x 96-well plate.

Component	Reaction Mix
250X AbRed Stock Solution	20 µL
50X HRP Stock Solution	100 µL
10X Glucose Stock Solution	500 µL
Assay Buffer	4.3 mL
TOTAL VOLUME	5 mL

12.2.2 Add 50 µL of Glucose Oxidase reaction mix into each well to make the total assay volume of 100 µL. Mix well.

12.3 Measurement:

12.3.1 Monitor absorbance increase at OD 570 nm on a microplate reader in kinetic mode, every 2-3 minutes, for at least 10-30 minutes at 37°C protected from light.

Δ Note: Incubation time depends on the glucose oxidase activity in the samples. We recommend measuring OD in a kinetic mode. Use longer incubation time to increase sensitivity (detect lower range of GOX), and shorter incubation time for higher range of GOX activity.

13. Data Analysis

– 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 readings for each blank (Standard #8), standard, and samples.

13.2 Subtract the mean absorbance value of the blank from all standard and sample readings. This is the corrected absorbance.

13.3 Select a time T in the linear phase of the kinetics. The same time point will be used for all conditions for the next steps of the analysis.

13.4 Plot the standard curve readings using the corrected absorbance and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step).

13.5 Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Use this equation to calculate the Glucose Oxidase activity in the samples, using the OD value of the samples at time T.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

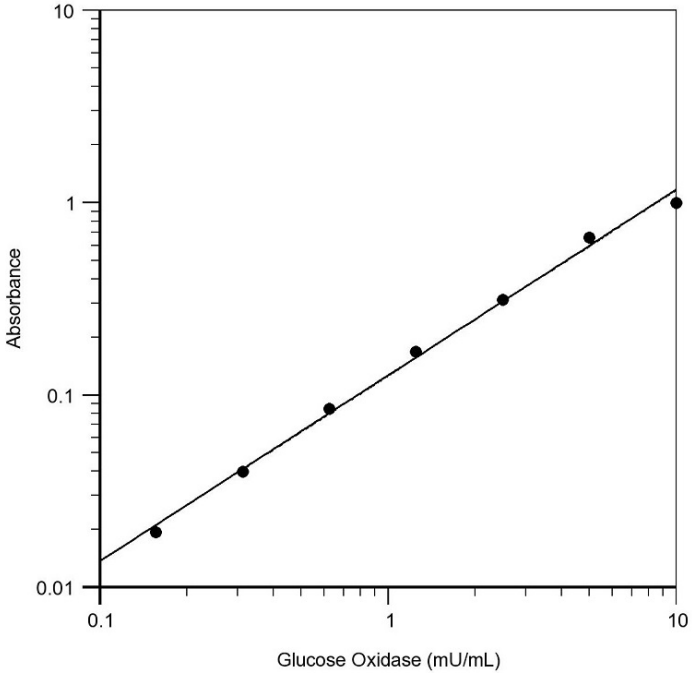


Figure 1. Typical glucose oxidase dose response curve. Absorbance was measured on a 96-well clear bottom plate using a SpectraMax microplate reader (Molecular Devices) with path check on. As low as 12.5 mU/mL glucose oxidase activity can be detected after 30 minutes incubation.

15. Quick Assay Procedure

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

- Prepare reagents and aliquot; get equipment ready.
- Prepare Glucose Oxidase standard dilution [0.156-10 mU/mL].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL) and samples (50 µL).
- Prepare Glucose oxidase reaction mix:

Component	Reaction Mix
250X AbRed Stock Solution	20 µL
50X HRP Stock Solution	100 µL
10X Glucose Stock Solution	500 µL
Assay Buffer	4.3 mL
TOTAL VOLUME	5 mL

- Add 50 µL of Glucose Oxidase reaction mix into each well.
- Monitor absorbance increase at OD 570 nm on a microplate reader in kinetic mode for 10-30 minutes at 37°C protected from light.

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. Notes

Technical Support

For all technical and commercial enquires please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)