BOC

REDOX TECHNOLOGIES

KB03040

Glucose Oxidase Activity Assay Kit

96 well plate
100/200/400 tests

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1. General information

PRECAUTIONS

Please read this manual carefully before beginning the assay.

This product is designed for **research use only**. It is not approved for human or animal use or clinical diagnosis. All chemicals should be handled with care and in accordance with laboratory safety practices. It is recommended to use basic Personal Protective Equipment.

Do not use after the expiration date stated on the packaging.

Do not mix or substitute reagents or materials from other kit batches or vendors.

For the **material safety data sheet** (MSDS) please contact us at info@bioquochem.com

TECHNICAL RECOMMENDATIONS

Store reagents as indicated in **Materials and storage** section.

Be sure to keep the bottle capped when not in use.

Let the components reach room temperature (RT) before use.

Immediately before use, gently invert and rotate reagent bottles several times to mix the contents thoroughly.

Avoid foaming or bubbles when mixing or reconstituting components.

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

Be sure to use the optimal microplate for the assay. Flat bottom transparent microplates for UV/VIS applications, and black microplates for fluorescence measurements.

2. Technical specifications

Available sizes

100/200/400 tests

Required sample volume

50 µL/test

Compatible samples

Animal and plant tissue homogenates, cells, plasma, serum, urine and other biological fluids

Type of detection

Colorimetric (580 nm)

3. Materials and storage

MATERIALS SUPPLIED

Store kit components as indicated below:

Item	No. Tests	Units	Storage
Reagent A	100	1	4 °C
	200	2	
	400	4	
Reagent B	100	1	-20 °C
	200	2	
	400	4	
Reagent C	100	1	-20 °C
	200	2	
	400	4	
Reagent D	100	1	-20 °C
	200	2	
	400	4	
Standard	100	1	-20 °C
	200	2	
	400	4	
Transparent 96-Well Microplate	100	1	RT
	200	2	
	400	4	

MATERIALS NEEDED BUT NOT SUPPLIED

- Double distilled water (ddH_2O) as Milli-Q Ultrapure Water
- Incubator
- Labware materials (micropipettes, tubes, stirring/mixing equipment)
- Colorimetric microplate reader – equipped with filter for OD 580 nm

STORAGE CONDITIONS

On receipt, store kit components as indicated above. Under these conditions, the reagents are stable in the original packaging until the expiration date indicated on the outside of the box. **Reagent C, Reagent D and Standard are light sensitive** and should be stored in the dark. Standard solutions are unstable in the presence of oxygen. Prepare a fresh set of standards for every use.

4. Introduction

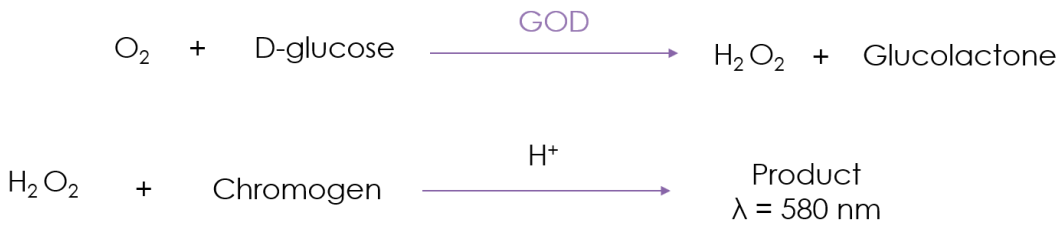
Glucose Oxidase (GOD) (EC 1.1.3.4) is present in certain species of insects, fungi and bacteria and catalyzes the oxidation of β-D-glucose into hydrogen peroxide and D-glucono-1,5-lactone, transforming sugars into metabolites.

GOD is commonly used for glucose determination in biosensors, in body fluids and in removing residual glucose and oxygen from beverages, food and related products.

BQC Glucose Oxidase Activity Assay Kit is a quick, easy, and reproducible assay to determine GOD activity in a wide variety of samples.

5. Assay Principle

This kit is based on the oxidation of D-glucose by GOD that produces hydrogen peroxide (H_2O_2), which under acid condition reacts with a chromogen to obtain a product that can be measured at 580 nm. Therefore, GOD activity in the sample is proportional to the product formed.



Glucose Oxidase Activity Assay Principle

6. Assay preparation

REAGENT PREPARATION

All assay reagents not listed below are ready to use as supplied. Allow the reagents to reach room temperature before use.

Working Buffer: make a 1:20 dilution of **Reagent A** with deionized water (for 100 tests, mix 1 mL of Reagent A with 19 mL deionized water).

> **CAUTION:** Working Buffer is stable for two months at 4 °C.

Positive Control: Reagent D vial contains Glucose Oxidase, which is used as positive control. Take 2 µL of **Reagent D** and mix it with 998 µL of **Working Buffer**. Protect it from light and place it on ice to be tested.

> **CAUTION:** Positive control must be freshly prepared and used immediately. Store unsused Reagent D at -20 °C.

Standard Solution (H_2O_2):

- Prepare 10 mM of H_2O_2 Standard by mixing 10 µL of **Standard** with 870 µL of **Working Buffer**.
- Prepare 100 µM of H_2O_2 Standard by mixing 10 µL of the previous solution with 990 µL of **Working Buffer.**
- Prepare 40 µM of H_2O_2 Standard by mixing 40 µL of the previous solution with 960 µL of **Working Buffer** to obtain the **Standard Solution 40 µM** for the standard calibration curve.

> **CAUTION:** Standard Solution must be freshly prepared and used immediately. Store unsused Standard at -20 °C.

STANDARD CALIBRATION

Prepare H_2O_2 standards for the calibration curve from the Standard Solution according to the following Table. Prepare the standards immediately prior to each assay. Vortex tubes thoroughly. Discard standard solutions after use.

Standard	Standard Solution 40 µM (µL)	Working Buffer (µL)	Standard Concentration (µM H_2O_2)
Std 1 (Reagent Blank)	0	200	**0**
Std 2	5	195	**1**
Std 3	10	190	**2**
Std 4	25	175	**5**
Std 5	50	150	**10**
Std 6	100	100	**20**
Std 7	150	50	**30**
Std 8	200	0	**40**

PLATE SET UP

BQC recommends running the standards, positive control and samples at least in duplicate (triplicate recommended). There is no specific pattern for using the wells on the plate. A proposed layout of standards (Std), positive control (PC) and samples (S) to be measured in duplicate is shown below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	PC	PC	S8	S8	S16	S16	S24	S24	S32	S32
B	Std 2	Std 2	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
C	Std 3	Std 3	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
D	Std 4	Std 4	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
E	Std 5	Std 5	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
F	Std 6	Std 6	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
G	Std 7	Std 7	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
H	Std8	Std8	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39

Example of plate layout for the GOD Activity Assay Kit

7. Sample preparation

The following sample preparation protocols are intended as a guide only. The optimal conditions for sample preparation must be determined by the end user. It is recommended to use fresh samples. If it is not possible, aliquot and store samples with minimal freeze/thawing.

BQC GOD Activity Assay Kit can be used to detect GOD activity in a wide variety of cells, animal tissue, and biological fluid samples.

Animal Tissues. Perfuse tissue with cold PBS to remove any red blood cells. Weigh 0.1 g of tissue and mix with 1 mL of cold buffer. Centrifuge at 12000 g for 5 minutes at 4 °C. Use the supernatant for the assay and place on ice to be tested.

Cells and bacteria. Collect $5 \cdot 10^6$ cells, wash with cold PBS and homogenize with 1 mL of cold buffer. Centrifuge at 12000 g for 5 minutes at 4 °C. Use supernatant for the assay and place it on ice to be tested.

Serum, plasma, and other biological fluids can be tested directly.

Reagents and materials required for sample preparation are not supplied with the kit. Before doing sample preparation, consider the volume of sample required per test; see **Technical specifications** section.

It is recommended to assay different sample dilutions to ensure the values fall within the standard curve.

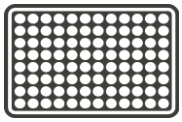
8. Assay protocol

Prepare and mix all reagents thoroughly before use. Each standard, sample or positive control should be assayed at least in duplicate.

1  **Standard**, **sample** and **positive control** wells: add **50 µL** of **sample**, **standard** or **positive control** to each tube

2  Add **50 µL** of **Reagent B** to each tube

3  **Incubate** the reaction for **2 minutes** at **37 °C** in the **dark**

4  Set up the plate design

5  Transfer **60 µL** of the mixture from each tube to a 96-well plate

6  Add **40 µL** of **Reagent C** to each well

7  Read the **absorbance** of all wells at **580 nm** and record the value as $A_{t0'}$

8  **Incubate** the reaction for **10 minutes** at **37 °C** in the **dark**

9  Read the **absorbance** of all wells at **580 nm** and record the value as $A_{t10'}$

If you need to **adapt this kit** for another form of the assay (for example cuvette), **contact us at** info@bioquochem.com

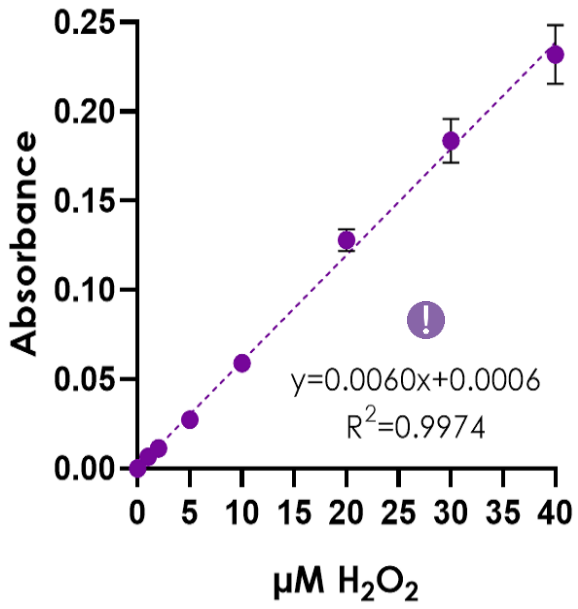
9. Data analysis

ANALYSIS OF THE STANDARDS

- Subtract the initial absorbance ($A_{t0'}$) from the absorbance measured after ten minutes ($A_{t10'}$) for each standard well:

$$\Delta A = A_{t10'} - A_{t0'}$$

- Calculate the average of ΔA for each standard.
- Subtract the average of ΔA of the blank (Std 1) from the average of ΔA of each standard to obtain the blank-corrected absorbance of the standards.
- Create a standard curve by plotting the blank-corrected absorbance of the standards as a function of the standard concentration (see ¡Error! No se encuentra el origen de la referencia. section). A typical standard curve (**y** = slope ·**x** ± intercept) for this assay is shown below.



Standard curve for Glucose Oxidase Activity Assay Kit

This standard curve is an example of the data typically obtained with this kit. **DO NOT USE** this standard curve to calculate the GOD activity of your samples. A new standard curve must be performed by the end user.

ANALYSIS OF THE SAMPLES

- Subtract the initial absorbance ($A_{t0'}$) from the absorbance measured after ten minutes ($A_{t10'}$) for each sample and positive control well:

$$\Delta A = A_{t10'} - A_{t0'}$$

- Calculate the average of ΔA for each sample and positive control.
- Subtract the average of ΔA of the blank (Std 1) from the average of ΔA of each standard and positive control to obtain the blank-corrected absorbance of the standards and positive control (**ΔA_s**).
- Calculate the H_2O_2 concentration using the following formula. Slope and intercept values are obtained from the standard curve.

$$H_2O_2\ (\mu M) = \left(\frac{\Delta As - intercept}{slope} \right)$$

To convert the H_2O_2 concentration obtained to the activity of GOD, use the following formula:

- **Calculated by liquid sample**:

$$GOD\ (U/mL) = H_2O_{2(\mu M)}$$

- **Calculated by fresh weight**:

$$GOD\ (U/g) = \frac{H_2O_{2(\mu M)} \times 0.05}{W}$$

- **Calculated by cell number or bacteria**:

$$GOD\ (U/10^4\ cells) = H_2O_{2(\mu M)} \times 0.0001$$

- **Calculated by protein concentration**:

$$GOD\ (U/mg\ protein) = \frac{H_2O_{2(\mu M)} \times 0.05}{Cp}$$

Where W is the sample weight (g) and Cp is the sample protein concentration (mg/mL).

One Unit (U) of GOD is defined as the catalytic activity responsible of the production of 1 nmol of H_2O_2 in 1 minute per mL of liquid sample, 1 g of tissue, 10^4 cells or 1 mg of tissue protein, depending on the formula used.

When working with diluted samples the concentration values obtained must be multiplied by the dilution factor to obtain the enzymatic activity value of the undiluted sample.

10. Troubleshooting

This troubleshooting table provides potential sources and solutions for common problems observed with BQC Assay Kits. **The problems listed below could occur when using any BQC Assay Kit**. They are not specific for this Assay Kit.

Problem	Possible Cause	Recommended Solution
Wells have color but there is no reading	Plate read at incorrect wavelength	Check the wavelength used in the assay
	Incorrect microplate	Use the correct microplate for your application UV/Vis: transparent Fluorescence: black wells/transparent bottom
Standard readings do not follow a linear pattern	Pipetting errors in preparation of standards	Avoid pipetting small volumes (<5 µL) Be careful not to splash from well to well
	Air bubbles formed in well(s)	Use reverse pipetting technique
	Standard stock is at incorrect concentration	Always refer to dilutions described in **Assay preparation**
	Improperly thawed reagents	Thaw all components completely and mix well before use
	Use of improperly stored reagents	Store the components appropriately Use fresh components from the standard curve
	Incorrect incubation times or temperatures	Refer to **Refer to Assay protocol**
Dispersion of standard and sample readings	Pipetting errors	Avoid pipetting small volumes (<5 µL) Be careful not to splash from well to well
	Air bubbles formed in well(s)	Use reverse pipetting technique

Problem	Possible Cause	Recommended Solution
Sample erratic values	Samples contain interfering substances	Dilute sample further (if possible)
	Inappropriately stored samples or samples used after multiple freeze-thaw cycles	Use fresh samples or store appropriately until use
	Samples not deproteinized	Use an appropriate deproteinization protocol
	Cells/Tissue samples not homogenized completely	Repeat the sample homogenization
	Inappropriate sample dilution buffer	Refer to **Assay preparation**
Sample reading fall outside the detection range	Samples are too diluted/concentrated No analyte/activity is observed in the sample	Re-assay using different sample dilutions

STILL HAVING PROBLEMS?

Contact BQC if you have any further questions, our team will be pleased to help you:

	Phone	+ 34 985 26 92 92
	E-mail	info@bioquochem.com
	Business hours	Monday-Thursday: 8.30 to 17.00 (CEST) Friday: 8.00 to 15.00 (CEST)

11. Additional information

BQC Glucose Oxidase Activity Assay Kit is a quick (< 15 minutes) assay for determining GOD Activity in a wide variety of samples with good reproducibility (RSD < 5%).

H_2O_2 concentration obtained can be converted to GOD activity with a formula based on sample type used. See **Data analysis** section.

If unexpected results are obtained running your samples, please contact us at info@bioquochem.com

12. Related products

More products available on bioquochem.com

Reference	Product
KB03047	Peroxidase Activity Assay Kit
KB03011	Superoxide Dismutase Activity Assay Kit
KB03050	Thioredoxin Peroxidase Activity Assay Kit

13. Warranties and limitation of liability

BQC shall not in any event be liable for incidental, consequential or special damages of any kind resulting from any use or failure of the products, even if BQC has been advised of the possibility of such damage including, without limitation, liability for loss of use, loss of work in progress, downtime, loss of revenue or profits, failure to realize savings, loss of products of buyer or other use or any liability of buyer to a third party on account of such loss, or for any labor or any other expense, damage or loss occasioned by such product including personal injury or property damage is caused by BQC´s gross negligence. Any and all liability of BQC hereunder shall be limited to the amounts paid by the buyer for the product.

Buyer´s exclusive remedy and BQC´s sole liability hereunder shall be limited to a refund of the purchase price, or the replacement of all material that does not meet our specifications.

Said refund or replacement is conditioned on buyer giving written notice to BQC within 30 days of shipment.

Expiration date: 1 year from the date of fabrication. Expiration date is indicated on the outside of the box.

For further details, please refer to our website **bioquochem.com**

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