GLUCOSE OXIDASE (GOD), LYOPHILIZED

INSTRUCTIONS FOR USE

1. PRODUCT INFORMATION

Catalog Number	EV-OXR-002
Product Name	Glucose Oxidase (GOD), Lyophilized
Category	Oxidoreductase
Pack Size	1g/bottle
Regulatory Status	For Research Use Only (RUO)
OEM Reference	GPE008001
Version	1.0
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2. INTENDED USE

Glucose Oxidase (GOD), Lyophilized is a recombinant flavoprotein oxidoreductase that catalyzes the oxidation of β-D-glucose to D-glucono-delta-lactone and hydrogen peroxide using molecular oxygen as the electron acceptor. This enzyme is supplied as lyophilized powder with specific activity ≥50 U/mg dry weight and is intended for use in enzymatic determination of D-glucose in research assays, glucose biosensor development for continuous monitoring applications, fermentation process control, and food science research including oxygen depletion systems to prevent non-enzymatic browning. The homodimeric enzyme (approximately 160 kDa) contains FAD and Fe cofactors and exhibits optimal activity at pH 4.5 and 42°C, with activity maintained across pH 4-7 and temperature range 40-50°C. For Research Use Only. Not for use in diagnostic procedures.

3. KIT COMPONENTS

Component	Quantity / Volume	Storage
Glucose Oxidase, Lyophilized Powder	1 g	-20°C
Reconstitution Buffer (50 mM Sodium Phosphate, pH 5.1)	10 mL	2-8°C
β-D-Glucose Reference Standard (100 mM)	1 mL	2-8°C
Stabilizer Solution (Glycerol-based, 50% v/v)	5 mL	2-8°C
Product Information Sheet	1 sheet	Room temperature

4. MATERIALS REQUIRED BUT NOT PROVIDED

- β-D-Glucose substrate (minimum 100 mM stock solution recommended)
- Phosphate buffer, pH 5.1 or pH-adjusted buffer system (50-100 mM for activity assays)
- Molecular oxygen or oxygen-saturated reaction buffer (for maximum activity enhancement)
- Horseradish peroxidase (HRP) for coupled colorimetric glucose determination assays
- Chromogenic substrate for peroxidase reaction (e.g., o-dianisidine, ABTS, or 4-aminoantipyrine with phenol)
- Temperature-controlled reaction vessel or spectrophotometer with temperature control (35-42°C)

- Ultrapure water (18.2 MΩ·cm) for reconstitution of lyophilized powder
- Standard glucose calibration solutions (0-50 mM range) for quantitative assay validation

5. STORAGE AND STABILITY

Storage Temperature	-20°C recommended for lyophilized powder
Appearance	Yellow lyophilized powder
Shelf Life	12 months from manufacture date
Shipping Conditions	On dry ice
Freeze-Thaw Cycles	Maximum 3 cycles recommended
Working Solution	Stable on ice for up to 8 hours

6. PRECAUTIONS AND WARNINGS

- For Research Use Only. Not for use in diagnostic procedures.
- Avoid repeated freeze-thaw cycles. Aliquot reagents if needed.
- Handle all reagents on ice. Return to -20°C storage immediately after use.
- Wear appropriate PPE: gloves, lab coat, and eye protection at all times.
- Dispose of waste in accordance with local, state, and federal regulations.
- Do not use reagents past their expiry date.

7. PROTOCOL

GLUCOSE OXIDASE (GOD) LYOPHILIZED – DETAILED PROTOCOL

Product: Glucose Oxidase, Recombinant, Diagnostic Reagent Grade

Catalog Number: GPE008001

Enzyme Classification: Oxidoreductase (EC 1.1.3.4)

Specific Activity: ≥45 U/mg lyophilized powder

Source: Recombinant expression in yeast using cloned Aspergillus niger glucose oxidase gene

PRINCIPLE

Glucose oxidase is a homodimeric flavoprotein (approximately 160 kDa) consisting of two identical 80 kDa subunits linked by disulfide bonds. Each subunit contains one mole FAD (flavin adenine dinucleotide) and one mole Fe as cofactors. The enzyme catalyzes the oxidation of β-D-glucose to D-glucono-delta-lactone and hydrogen peroxide using molecular oxygen as the electron acceptor. This reaction is highly specific for β-D-glucose and produces stoichiometric amounts of hydrogen peroxide, which can be coupled with peroxidase reactions for colorimetric detection in diagnostic assays, glucose biosensors, and food processing applications.

MATERIALS REQUIRED

Enzyme: Glucose Oxidase lyophilized powder (≥45 U/mg)

Substrate: β-D-glucose (anhydrous, ACS reagent grade)

Buffer: 0.1 M sodium acetate buffer, pH 5.1 (for unit definition assays) or pH 4.5 (for optimal activity)

Coupling enzyme (optional): Horseradish peroxidase for colorimetric detection

Chromogen (optional): o-Dianisidine, ABTS, or other peroxidase substrate

Deionized water (18.2 MΩ·cm)

Temperature-controlled water bath or heat block (35°C for unit definition, 42°C for optimal activity)

Spectrophotometer (420-560 nm depending on chromogen)

Microcentrifuge tubes or reaction vessels

pH meter

Oxygen source (optional, for activity enhancement)

SAFETY PRECAUTIONS

Wear appropriate personal protective equipment including laboratory coat, safety glasses, and nitrile gloves.

Handle lyophilized enzyme powder in a fume hood or biosafety cabinet to prevent inhalation.

Some chromogenic substrates (e.g., o-dianisidine) are potential carcinogens; consult SDS before use.

Hydrogen peroxide is a strong oxidizer; handle with care and dispose according to institutional guidelines.

This product is For Research Use Only (RUO). Not for human or animal therapeutic or diagnostic use.

PROTOCOL STEPS

1. RECONSTITUTION OF LYOPHILIZED GLUCOSE OXIDASE

1. Remove lyophilized glucose oxidase vial from -20°C storage and allow to equilibrate to room temperature (20-25°C) for 10-15 minutes before opening to prevent moisture condensation.

2. Briefly centrifuge the vial (2-3 seconds at 1000 × g) to collect powder at the bottom.

3. Calculate the volume of reconstitution buffer needed based on desired final enzyme concentration. For general applications, reconstitute to 10-50 U/mL in 0.1 M sodium acetate buffer, pH 5.1.

4. Add the calculated volume of ice-cold 0.1 M sodium acetate buffer (pH 5.1) dropwise to the lyophilized powder.

5. Gently swirl or tap the vial to mix. Do not vortex vigorously as this may denature the enzyme or cause foaming.

6. Incubate on ice for 5-10 minutes to allow complete dissolution.

7. If undissolved particles remain, centrifuge at 10,000 × g for 2 minutes at 4°C and transfer the supernatant to a fresh tube. Discard any precipitate.

8. Measure protein concentration using Bradford or BCA assay if needed to confirm specific activity calculation.

2. PREPARATION OF REACTION BUFFER (pH 5.1 FOR UNIT DEFINITION ASSAY)

9. Prepare 0.1 M sodium acetate buffer by dissolving 8.2 g sodium acetate trihydrate in 800 mL deionized water.

10. Adjust pH to 5.1 using glacial acetic acid while monitoring with a calibrated pH meter.

11. Bring final volume to 1000 mL with deionized water.

12. Filter through 0.22 µm membrane filter and store at 4°C for up to 1 month.

3. PREPARATION OF SUBSTRATE SOLUTION

13. Prepare 1 M β-D-glucose stock solution by dissolving 18.02 g anhydrous D-glucose in 0.1 M sodium acetate buffer (pH 5.1) to a final volume of 100 mL.

14. Allow the solution to mutarotate at room temperature for at least 2 hours (preferably overnight at 4°C) to reach equilibrium between α and β anomers. This ensures consistent substrate availability.

15. For working substrate solution, dilute stock to 0.1 M (10% w/v) in 0.1 M sodium acetate buffer, pH 5.1.

16. Warm substrate solution to reaction temperature (35°C for unit definition or 42°C for optimal activity) before use.

4. ENZYME ACTIVITY ASSAY (UNIT DEFINITION METHOD AT 35°C, pH 5.1)

17. Pre-warm water bath or heat block to 35°C (±0.5°C).

18. Prepare reaction mixture in a cuvette or microplate well: Add 2.7 mL of 0.1 M sodium acetate buffer (pH 5.1, pre-warmed to 35°C).

19. Add 0.2 m

8. EXPECTED RESULTS

Expected Results

When stored and handled according to instructions, Glucose Oxidase (GOD) demonstrates specific activity ≥50 U/mg dry weight, meeting or exceeding the specified performance criterion of ≥45 U/mg lyophilized powder. The enzyme exhibits high specificity for β-D-glucose oxidation at pH 4.5–5.1 and 40–50°C, producing stoichiometric amounts of D-glucono-δ-lactone and hydrogen peroxide with molecular oxygen as the electron acceptor. Lyophilized powder remains stable at −20°C with full retention of catalytic activity; reconstituted enzyme solutions should be used immediately or stored according to experimental requirements to maintain optimal performance in diagnostic glucose assays, biosensor applications, and food preservation systems.

9. TROUBLESHOOTING GUIDE

For troubleshooting assistance, contact techsupport@enzovera.com

10. DOCUMENT CONTROL

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