GLUCOSE DEHYDROGENASE (GDH-FAD), LYOPHILIZED

INSTRUCTIONS FOR USE

1. PRODUCT INFORMATION

Catalog Number	EV-OXR-001
Product Name	Glucose Dehydrogenase (GDH-FAD), Lyophilized
Category	Oxidoreductase
Pack Size	500KU/bottle, 10g/bottle
Regulatory Status	For Research Use Only (RUO)
OEM Reference	GPE006001
Version	1.0
Issue Date	2026-05-14

2. INTENDED USE

Glucose Dehydrogenase (FAD-dependent) catalyzes the oxidation of β-D-glucose to gluconic acid and hydrogen peroxide, requiring FAD as a cofactor. This enzyme exhibits high specificity for β-D-glucose with low cross-reactivity toward other monosaccharides and maintains thermal stability without producing precipitation or turbidity after reaction completion. Applications include glucose determination in clinical diagnostics, biosensor development, enzymatic fuel cells, synthesis of chiral molecules, and food and beverage preservation including oxygen removal in packaging systems and prevention of beer aging. For Research Use Only. Not for use in diagnostic procedures.

3. KIT COMPONENTS

Component	Quantity / Volume	Storage
Glucose Dehydrogenase (FAD-dependent) Lyophilized Powder	500 KU (≥300 U/mg specific activity)	-20°C or lower in a non-frost-free freezer
Reconstitution Buffer (10×)	10 mL	2–8°C
FAD Cofactor Solution (10 mM)	1 mL	-20°C, protect from light
Glucose Standard Solution (100 mM)	1 mL	2–8°C
Stabilization Buffer (10×)	5 mL	2–8°C

4. MATERIALS REQUIRED BUT NOT PROVIDED

- β-D-glucose substrate (analytical grade or higher)
- FAD (flavin adenine dinucleotide) cofactor solution
- Phosphate buffer, pH 7.5, 50-100 mM
- Temperature-controlled water bath or incubator (37°C for assay; 40-50°C for optimal activity)
- Spectrophotometer capable of monitoring NADH or product formation at appropriate wavelength
- Ultrapure water (Type I, 18.2 MΩ·cm) for enzyme reconstitution
- Microcentrifuge tubes and pipettes for accurate dispensing
- Optional: Oxygen electrode or hydrogen peroxide detection system for activity monitoring

5. STORAGE AND STABILITY

Storage Temperature	-20°C or lower in a non-frost-free freezer; avoid repeated freeze-thaw cycles
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 DEHYDROGENASE (GDH-FAD) PROTOCOL

Enzovera Life Sciences

Catalog No. EZ-GDH-FAD-001

Lyophilized Oxidoreductase Enzyme

FOR RESEARCH USE ONLY

PRODUCT DESCRIPTION

Glucose Dehydrogenase (FAD-dependent) is a recombinant oxidoreductase enzyme that catalyzes the oxidation of β-D-glucose to gluconic acid and hydrogen peroxide, requiring FAD (flavin adenine dinucleotide) as a cofactor. This enzyme exhibits high substrate specificity for β-D-glucose with minimal activity toward other monosaccharides. The lyophilized preparation maintains specific activity ≥300 U/mg dry weight and purity >95% by SDS-PAGE analysis. Molecular weight is approximately 65 kDa. Applications include clinical diagnostic glucose testing, electrochemical biosensors, enzymatic biofuel cells, implantable cardiac monitoring devices, food and beverage preservation, oxygen scavenging systems, and chiral synthesis reactions.

ENZYME CLASSIFICATION

E.C. Number: 1.1.5.9

CAS Number: 9035-82-9

Enzyme Class: Oxidoreductase (FAD-dependent dehydrogenase)

Cofactor Requirement: FAD (flavin adenine dinucleotide)

SPECIFICATIONS

Specific Activity: ≥300 U/mg lyophilized powder at 37°C, pH 7.5

Enzyme Definition: One unit converts 1 micromole of β-D-glucose to gluconic acid and hydrogen peroxide per minute at pH 7.5 at 37°C

Purity: >95% by SDS-PAGE

Nuclease Contamination: None detected

Protease Contamination: None detected

Appearance: White to off-white lyophilized powder

Storage Temperature: -20°C or lower in non-frost-free freezer

OPTIMAL REACTION CONDITIONS

Temperature: 42°C (optimum); activity range 40–50°C

pH: 7.5 (optimal for activity assay and standard reactions)

Buffer System: Phosphate buffer, pH 7.5

Cofactor: FAD must be present in reaction mixture

STORAGE AND STABILITY

Store lyophilized powder at -20°C or lower in a non-frost-free freezer protected from light and moisture. Under these conditions, the enzyme remains stable for 24 months from date of manufacture. Once reconstituted, prepare working aliquots and store at -20°C. Avoid repeated freeze-thaw cycles as enzyme activity decreases with each cycle. For frequent use, prepare small-volume working stocks and keep at 4°C for up to 7 days.

PROTOCOL: RECONSTITUTION AND REACTION SETUP

MATERIALS REQUIRED (not supplied)

Ultrapure water (18.2 megohm-cm, nuclease-free)

Phosphate buffer, 50 mM, pH 7.5

β-D-Glucose substrate solution, 100 mM in phosphate buffer pH 7.5

FAD (flavin adenine dinucleotide), 1 mM stock solution

Microcentrifuge tubes (1.5 mL, low-retention)

Calibrated pipettes and aerosol-barrier tips

Water bath or heat block set to 42°C

Spectrophotometer capable of reading absorbance at 340 nm or 420 nm (depending on detection method)

Vortex mixer

Ice bucket

SECTION 1: RECONSTITUTION OF LYOPHILIZED ENZYME

1. Remove the vial of lyophilized Glucose Dehydrogenase (GDH-FAD) from -20°C storage and allow it to equilibrate to room temperature for 10 minutes. Do not open the vial until it reaches room temperature to prevent moisture condensation.

2. Briefly centrifuge the vial at 1000 × g for 10 seconds to collect all lyophilized powder at the bottom of the tube.

3. Determine the desired enzyme concentration for reconstitution. For a standard working stock of 10 mg/mL, add the appropriate volume of ice-cold phosphate buffer (50 mM, pH 7.5) to achieve this concentration. For example, if the vial contains 5 mg of lyophilized enzyme, add 500 microliters of buffer.

4. Add the calculated volume of ice-cold 50 mM phosphate buffer pH 7.5 dropwise to the inner wall of the vial to minimize foam formation. Do not pipette directly onto the lyophilized powder.

5. Allow the buffer to contact the powder for 2 minutes without agitation to enable initial hydration.

6. Gently swirl the vial for 30 seconds using circular motions. Avoid vigorous vortexing as this may denature the enzyme or create excessive foam.

7. Place the vial on ice for 5 minutes to allow complete dissolution. Gently invert the vial 5–6 times every 2 minutes during this period.

8. Inspect the solution visually to confirm complete dissolution. The solution should be clear to slightly opalescent with no visible particulates.

9. If particulates remain, centrifuge at 10,000 × g for 2 minutes at 4°C and transfer the supernatant to a fresh tube, leaving any insoluble material behind.

10. Measure the protein concentration of the reconstituted enzyme using a spectrophotometer at 280 nm or by Bradford/BCA assay if precise concentration is required for activity calculations.

SECTION 2: PREPARATION OF REACTION COMPONENTS

11. Prepare 50 mM phosphate buffer pH 7.5 by dissolving appropriate amounts of monobasic and dibasic sodium phosphate in ultrapure water and adjusting to pH 7.5 using a calibrated pH meter. Filter through a 0.22-micrometer membrane

8. EXPECTED RESULTS

When reconstituted and assayed under standard conditions (pH 7.5, 37°C with FAD cofactor), this recombinant FAD-dependent glucose dehydrogenase exhibits specific activity ≥300 U/mg dry weight, with one unit defined as the amount of enzyme that converts 1 µmole of β-D-glucose to gluconic acid and hydrogen peroxide per minute. The enzyme demonstrates >95% purity by SDS-PAGE analysis, high substrate specificity for β-D-glucose with minimal cross-reactivity to other monosaccharides, and thermal stability with optimum activity at 42°C. Quality control testing confirms absence of nuclease and protease contamination, ensuring suitability for biosensor fabrication, clinical glucose determination, and industrial glucose monitoring applications.

9. TROUBLESHOOTING GUIDE

For troubleshooting assistance, contact techsupport@enzovera.com

10. DOCUMENT CONTROL

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