Glucose Oxidase Assay Kit (Colorimetric/Fluorometric)

LS-K233-100 (100 Tests) • **Store at -20°C**

Introduction

Glucose oxidase catalyzes the oxidation of glucose from D-glucose to D-glucono-δ-lactone. Physiologically, it aids in the breakdown of glucose into smaller metabolites. It is widely used in electrochemical glucose sensors designed for diabetes patients. Simple, direct and high throughput assays for measuring glucose oxidase activity find wide applications in research and drug discovery. LSBio's glucose oxidase assay kit uses a single Working Reagent that combines the glucose oxidase reaction and color reaction in one step. The change in color intensity of the reaction product at 570 nm or fluorescence intensity at $\lambda_{ex/em}$ = 530/585 nm is directly proportional to glucose oxidase activity in the sample.

Key Features

- Sensitive and accurate. Use as little as 20 µL samples. Linear detection range in 96-well plate for 20 minute incubation at 25°C: 0.02 to 10 U/L glucose oxidase for colorimetric assays and 0.002 to 1.5 U/L for fluorometric assays.
- Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 20 min at room temperature.

Applications

- Direct Assays: glucose oxidase activity in cell lysate, culture medium and other biological samples.
- Drug Discovery/Pharmacology: effects of drugs on glucose metabolism.

Components

Component	K233-100 100 Tests
Assay Buffer	10 mL
Glucose (2M Glucose)	1.5 mL
HRP Enzyme	120 µL
Dye Reagent	120 µL
Standard (3% H_2O_2)	100 µL

Materials Not Supplied

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates, black 96-well plates and plate reader.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

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Assay Procedure

Colorimetric Procedure

Samples can be analyzed immediately after collection, or stored in aliquots at –20 °C. Avoid repeated freeze-thaw cycles. If particulates are present, centrifuge sample and use clear supernatant for assay.

1. Equilibrate all components to room temperature. During experiment, keep thawed Enzyme in a refrigerator or on ice.
2. H_2O_2 Standard Curve. Mix 5 µL 3% H_2O_2 and 914 µL dH_2O (final 4.8 mM) then mix 20 µL of the 4.8 mM H_2O_2 with 460 µL dH_2O to yield 200 µM H_2O_2. Prepare standards as shown in the Table below.

No	400 µM H_2O_2 + H_2O	Vol (µL)	H_2O_2 (µM)
1	100 µL + 0 µL	100	200
2	60 µL + 40 µL	100	120
3	30 µL + 70 µL	100	60
4	0 µL + 100 µL	100	0

Transfer 20 µL standards and samples into separate wells.

3. Working Reagent. Prepare bulk working reagent by mixing 75 µL Assay Buffer, 10 µL 2 M Glucose, 1 µL HRP Enzyme (vortex briefly before pipetting), and 1 µL Dye Reagent per reaction well in a clean tube. Transfer 80 µL Working Reagent into each reaction well. Tap plate to mix.
4. Read optical density immediately (OD_o) at 570 nm (550-585 nm). Incubate 20 min at room temperature, and then read optical density again (OD_{20}).

Fluorometric Procedure

For fluorometric assays, the linear detection range is 0.002 to 1.5 U/L glucose oxidase. Dilute the standards from Colorimetric Procedure 10X with dH_2O to obtain standards at 20, 12, 6 and 0 µM H_2O_2.

Transfer 20 µL standards and 20 µL samples into separate wells of a black 96-well plate.

Add 80 µL Working Reagent (see Colorimetric Procedure), tap plate to mix.

Read fluorescence immediately (F_o) at $\lambda_{ex/em}$ = 530/585 nm, incubate 20 min at room temperature, and then read fluorescence again (F_{20}).

Calculation

Subtract blank OD_{20} or F_{20} (water, #4) from all standard OD_{20} or F_{20} values and plot the ΔOD or ΔF against standard concentrations. Determine the slope using linear regression. Calculate the ΔOD_{Sample} or ΔF_{Sample} of all samples by subtracting OD0 or F0 from OD20 or F20 for each sample. Do the same for the blank (water, standard #4) to get ΔOD_{Blank} or ΔF_{Blank}.

Calculate the activity using the equation below:

$$\text{GO Activity} = \frac{\Delta R_{SAMPLE} - \Delta R_{BLANK}}{\text{Slope } (\mu M^{-1}) \bullet t} \times n \quad \text{(U/L)}$$

Where ΔR_{Sample} and ΔR_{Blank} are the change in optical density or fluorescent values of the sample and blank, respectively. Slope is the slope of the H_2O_2 standard curve, t is the incubation time (20 minutes), and n is the dilution factor.

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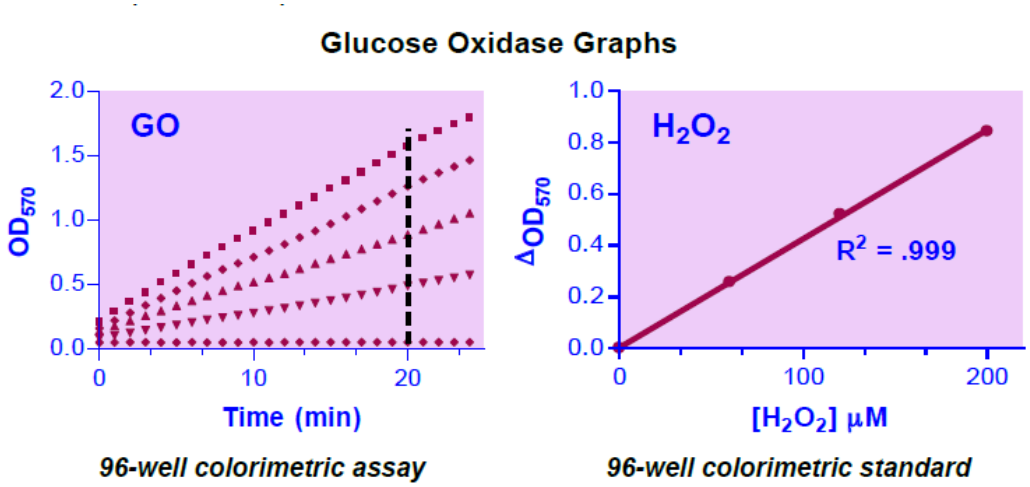
Notes: If the calculated sample glucose concentration is higher than 10 U/L in colorimetric assay or 1.5 U/L in fluorometric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor (n). For samples with low Glucose Oxidase activity, the incubation time can be increased.

Unit definition: 1 U/L of Glucose Oxidase catalyzes 1 µmole of H_2O_2 per minute at pH 7.0 and room temperature.

Sample Data



96-well colorimetric assay *96-well colorimetric standard*

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