Technical Manual

ColorFluor Glucose Oxidase Activity Assay Kit

Catalogue Code: BA0116

Pack Size: 100 assays

Research Use Only

DESCRIPTION

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. The Assay Genie ColorFluor Glucose Oxidase Activity 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 fluorimetric 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.

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer:	10 mL	**HRP Enzyme:**	120 µL
Glucose:	1.5 mL 2 M Glucose	**Dye Reagent:**	120 µL
Standard:	100 µL 3% H_2O_2		

Storage conditions. The kit is shipped on ice. Store assay buffer at 4°C and all other reagents at -20°C. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

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}).

FLUORIMETRIC PROCEDURE

For fluorimetric assays, the linear detection range is 0.002 to 1.5 U/L glucose oxidase. Dilute the standards from *Colorimetric Procedure* 10× 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 OD_0 or F_0 from OD_{20} or F_{20} 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:

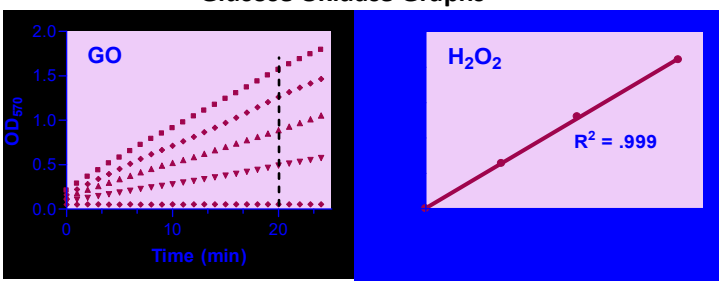
$$\text{GO Activity} = \frac{\Delta R_{SAMPLE} - \Delta R_{BLANK}}{\text{Slope } (\mu M^{-1}) \bullet t} \times n \quad \text{(U/L)}$$

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.

Notes: If the calculated sample glucose concentration is higher than 10 U/L in colorimetric assay or 1.5 U/L in fluorimetric 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.

Glucose Oxidase Graphs



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

MATERIALS REQUIRED, BUT NOT PROVIDED

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

LITERATURE

1. Raba J, Mottola HA. (1995). Glucose Oxidase as an Analytical Reagent. Critical Reviews in Analytical Chemistry 25(1):1-42.
2. Harris JM, Reyes C, Lopez GP. (2013). Common Causes of Glucose Oxidase Instability in in vivo Biosensing: a Brief Review. J Diabetes Sci Technol 7(4):1030-8.

3. Ferri S, Kojima K, Sode K. (2011). Review of glucose oxidases and glucose dehydrogenases: a bird's eye view of glucose sensing enzymes. J Diabetes Sci Technol 5(5):1068-76.

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