Glucose Oxidase Activity Assay Kit (Colorimetric)

Glucose Oxidase Activity Assay Kit (Colorimetric) can be used to measure Glucose Oxidase activity in cell / tissue lysate and cell culture supernatants.

Catalog number: ARG82162

Package: 100 tests

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

The glucose oxidase enzyme (GOx or GOD) also known as notatin (EC number 1.1.3.4) is an oxidoreductase that catalyses the oxidation of glucose to hydrogen peroxide and D-glucono-δ-lactone. This enzyme is produced by certain species of fungi and insects and displays antibacterial activity when oxygen and glucose are present.

Glucose oxidase is widely used for the determination of free glucose in body fluids (medical testing), in vegetal raw material, and in the food industry. It also has many applications in biotechnologies, typically enzyme assays for biochemistry including biosensors in nanotechnologies. It was first isolated by Detlev Müller in 1928 from Aspergillus niger. [Provide by Wikipedia: Glucose oxidase]

PRINCIPLE OF THE ASSAY

This Glucose oxidase Activity Assay Kit (Colorimetric) is a simple assay that measures the amount of glucose oxidase present in biological samples. This 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 λex/em = 530/585 nm is directly proportional to glucose oxidase activity in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
HRP Enzyme	120 µL	-20°C
Glucose (2 M)	1.5 mL	-20°C
Dye Reagent	120 µL	-20°C
Standard (3% H_2O_2)	100 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 570 nm
- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 585 nm
- Centrifuge and centrifuge tube
- Clear or black flat-bottom 96 well microplate
- Deionized or distilled water
- Pipettes, pipette tips and multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell or tissue lysate: Collect cell or tissue into centrifuge tube, discard the supernatant after centrifugation, add 1 mL of 1X PBS for 5×10^6 cell or 0.1 g tissue, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 10,000 x g for 20 minutes at 4°C, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Cell culture supernatant: To remove insoluble particles, centrifuge at 10,000 x g for 20 minutes at 4°C. The supernatant should be assayed directly.

Note:

- Samples can be analyzed immediately after collection, or stored in aliquots at-20°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Working Reagent:** for each reaction, mixing 75 µL of Assay Buffer, 10 µL of 2 M Glucose, 1 µL of HRP Enzyme (votex briefly before pipetting) and 1 µL of Dye Reagent. Prepare immediately before assay.
- **Standards:** Mix 5 µL of 3% H_2O_2 and 914 µL of distilled water (final 4.8 mM) then mix 20 µL of the 4.8 mM H_2O_2 with 460 µL of distilled water to yield 200 µM H_2O_2. Prepare standards as shown in the Table below.

Standard tube	H_2O_2 (µM)	Distilled water (µL)	Premix 200 µM H_2O_2 (µL)
S1	200	0	100
S2	120	40	60
S3	60	70	30
S4	0	100	0

ASSAY PROCEDURE

Equilibrate reagents to room temperature. Briefly centrifuge tubes before use.

COLORIMETRIC PROCEDURE

	Standard wells	Sample wells
Standard	20 µL	
Sample		20 µL
Working Reagent	80 µL	80 µL
Tap plate to mix well. Read the absorbance immediately (OD_0) at O.D. 570 nm (550-585 nm). Incubate for 20 minutes at room temperature, and then read the absorbance 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 10X with distilled water to obtain standards at 20, 12, 6 and 0 µM H_2O_2.

	Standard wells	Sample wells
Standard	20 µL	
Sample		20 µL
Working Reagent	80 µL	80 µL
Tap plate to mix well. Read fluorescence immediately (F_0) at λex/em = 530/585 nm. Incubate 20 minutes at room temperature, and then read fluorescence again (F_{20}).		

CALCULATION OF RESULTS

1. Subtract blank OD_{20} or F_{20} (distilled water, S4) 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 (distilled water, S4) to get ΔOD_{Blank} or ΔF_{Blank}. Calculate the activity using the equation below:

   GO Activity (U/L) = $[(\Delta R_{Sampe} - \Delta R_{Blank}) / (Slope, \mu M^{-1} \times t)] \times n$

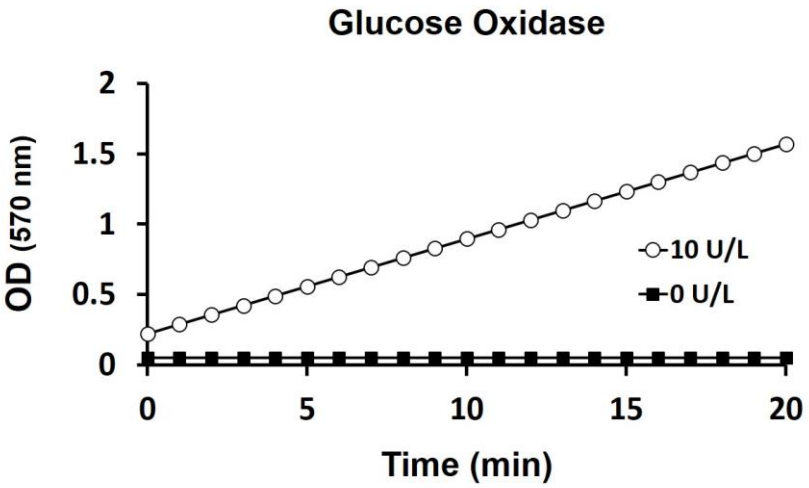
   Note:

   - ΔR_{Sampe} and ΔR_{Blank}: 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.

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

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Glucose Oxidase Activity Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

OD: 0.02 U/L; FL: 0.002 U/L