Glucose Oxidase Activity Assay Kit

Convenient kit for sensitive colorimetric/fluorometric detection of glucose oxidase activity in a variety of samples

Instruction Manual

Catalog Number	PK-CA577-K788
Description	The glucose oxidase enzyme (GOx) (EC 1.1.3.4) is an oxidoreductase commonly found in a wide variety of microorganisms that catalyzes the oxidation of glucose to hydrogen peroxide and D-glucono-δ-lactone. GOx aids in breaking the sugar down into its metabolites. PromoKine's Glucose Oxidase Activity Assay Kit provides a convenient tool for sensitive detection of the GOx in a variety of samples. Glucose oxidase in samples recognizes D-glucose as a specific substrate leading to proportional color development. The activity of GOx can be easily quantified colorimetrically (λ = 570 nm) or fluorometrically (Ex/Em = 535/585 nm). GOx assay detects glucose oxidase activity as low as 0.01mU.
Quantity	100 assays

Kit Components

Components	Quantity	Color Code
GOx Assay Buffer	25 ml	WM
OxiRed Probe	0.2 ml	Red
GOx Developer (lyophilized)	1 vial	Green
GOx Substrate	1 ml	Blue
H_2O_2 Standard (0.88 M)	0.1 ml	Yellow
GOx Positive Control (lyophilized)	1 vial	Purple

Storage and Reagents Preparation

Store kit at –20°C, protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Read entire protocol before performing assay.

GOx Developer and GOx Positive Control: Reconstitute with 220 µl Assay Buffer. Pipette up and down several times to completely dissolve the pellet (Don't vortex). Aliquot and freeze at -20°C. Stable for up to 2 months at -20°C after reconstitution or freeze-thaw cycles (< 5 times). Keep GOx Positive Control on ice while in use.

Assay Protocol

1. H_2O_2 Standard Curve: Add 10 µl 0.88 M H_2O_2 Standard to 870 µl dH_2O to make 10 mM H_2O_2 Standard. Dilute 10 mM H_2O_2 Standard further to 1:10 with Assay Buffer to make a 1 mM H_2O_2 Standard. Add 0, 2, 4, 6, 8, 10 µl of the diluted 1 mM H_2O_2 Standard into a series of wells of 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well H_2O_2 Standard.

For the fluorometric assay, dilute 1 mM H_2O_2 Standard 1:10 with Assay Buffer to make 100 µM H_2O_2 standard. Add 0, 2, 4, 6, 8, 10 µl of the diluted 100 µM H_2O_2 standard into a series of wells of 96-well plate to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well H_2O_2 Standard. Adjust the final volume to 50 µl with Assay Buffer.

2. Sample Preparations: Homogenize cells (1×10^6) with 100-200 µl Assay Buffer. Centrifuge at 13,000 g for 10 minutes to remove the insoluble material. 5-50 µl serum samples can be directly diluted in the Assay Buffer. Add 1-50 µl sample per well, adjust final volume to 50 µl with Assay Buffer. For samples having high background, prepare a parallel sample well as the background control. Note: For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.

3. Positive Control: Add 2-10 µl of Positive Control into the desired well(s) and adjust final volume to 50 µl with Assay Buffer.

4. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control
GOx Assay Buffer	36 µl	46 µl
GOx Developer	2 µl	2 µl
OxiRed Probe	2 µl	2 µl
GOx Substrate	10 µl	------

Add 50 µl of the reaction mix to each well containing samples, Positive Control, and standards. Mix well. Note: The fluorometric assay is ~10-fold more sensitive than the colorimetric assay so dilute the probe 10 times in Assay Buffer & use the same volume (2 µl). Background control mix is recommended for samples having high background.

5. Measurement: Incubate the plate for 5 minutes at 37°C and measure OD at 570 nm or fluorescence at Ex/Em = 535/585 nm (A1). Incubate for another 15 minutes to 2 hours at 37°C and again measure (A2). Note: Incubation time depends on the glucose oxidase activity in the samples. We recommend measuring in a kinetic method (preferably every 1–2 minutes) and choose the period of linear range to calculate the glucose oxidase activity of the samples. If the absorbance exceeds 0.7 OD at about ~15 minutes, dilute the sample and rerun the assay. The H_2O_2 Standard curve can read in end point mode (i.e. at the end of incubation time).

6. Calculation: Subtract the 0 standard reading from all readings. Plot H_2O_2 Standard Curve. Calculate the glucose oxidase activity of the test sample: **ΔOD = A2 – A1**. Apply the ΔOD to the H_2O_2 Standard Curve to get B nmol of H_2O_2 generated by Glucose Oxidase during the reaction time (ΔT = T2 – T1).

$$\text{Glucose Oxidase Activity} = \frac{B}{\Delta T \times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/ml}$$

Where: B is the H_2O_2 amount from Standard Curve (nmol).

ΔT is the time incubated (min).

V is the sample volume added into the reaction well (ml).

Unit Definition: One unit of GOx is the amount of enzyme that generates 1.0 µmol of H_2O_2 per min at 37°C.

Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

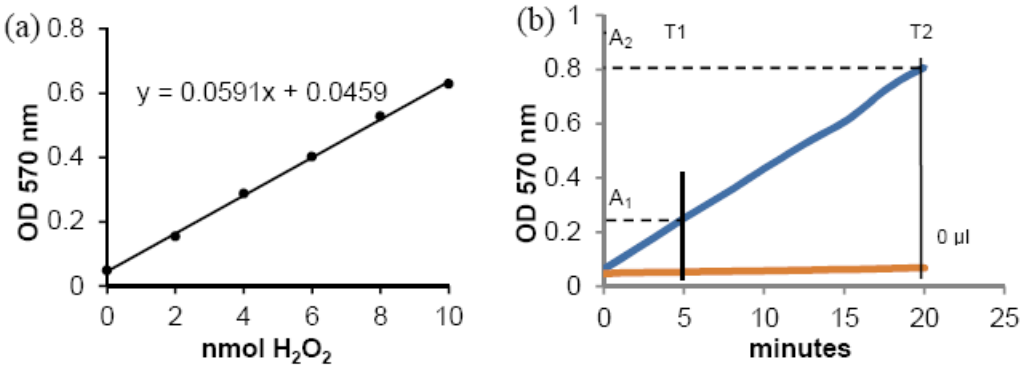

Figure: H_2O_2 Standard Curve (a). Glucose oxidase activity in sample (b). Assays were performed following the kit protocol.

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