Glucose Oxidase Activity Colorimetric/Fluorometric Assay Kit (#BN01001)

(Catalog #BN01001; 100 reactions; Store kit at –20°C)

I. Introduction:

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. Assay Genie's Glucose Oxidase Assay 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.

II. Kit Contents:

Components	BN01001	Cap Code	Part No.
GOx Assay Buffer	28 ml	WM	BN01001-1
GenieRed Probe	0.2 ml	Red	BN01001-2
GOx Substrate	1 ml	Blue	BN01001-3
GOx Developer	1 vial	Green	BN01001-4
GOx Positive Control	1 vial	Purple	BN01001-5
H_2O_2 Standard (0.88 M)	0.1 ml	Yellow	BN01001-6

III. Storage and Handling:

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

IV. Reagent Reconstitution and General Consideration:

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.

V. Glucose Oxidase 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 1:19 with Assay Buffer to make 0.5 mM H_2O_2 Standard. Add 0, 2, 4, 6, 8, 10 µl of the diluted 0.5 mM H_2O_2 Standard into a series of wells of 96-well plate to generate 0, 1, 2, 3, 4, 5 nmol/well H_2O_2 Standard. For the fluorometric assay, dilute 0.5 mM H_2O_2 Standard 1:10 with Assay Buffer to make 50 µM H_2O_2 standard. Add 0, 2, 4, 6, 8, 10 µl of the diluted 50 µM H_2O_2 standard into a series of wells of 96-well plate to generate 0, 0.1, 0.2, 0.3, 0.4, 0.5 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 min 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) & 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
GenieRed 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 min at 37°C & measure OD at 570 nm or fluorescence at Ex/Em = 535/585 nm (A_1). Incubate for another 15 minutes to 2 hrs at 37°C & again measure (A_2). **Note**: Incubation time depends on the glucose oxidase activity in the samples. We recommend measuring in a kinetic method (preferably every 1–2 min) and choose the period of linear range to calculate the glucose oxidase activity of the samples. If the absorbance exceeds 0.7 OD ~ 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: $\Delta OD = A_2 - A_1$. 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 ($\Delta T = T_2 - T_1$).

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

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.

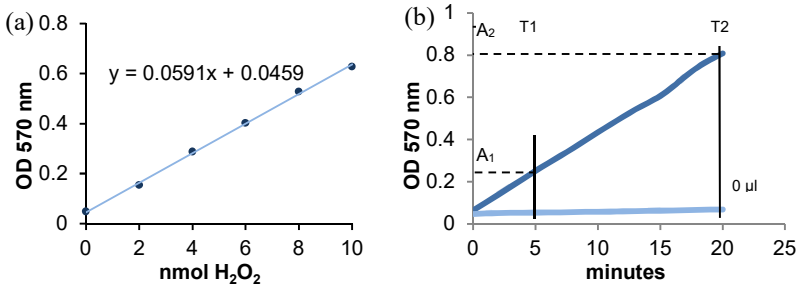

Figure: H_2O_2 Standard Curve (a). Glucose oxidase activity in sample (b). Assays were performed following kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		