



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. BioVision'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 0.01 mU.

II. Kit Contents:

Components	K788-100	Cap Code	Part No.
GOx Assay Buffer	25 ml	WM	K788-100-1
OxiRed™ Probe	0.2 ml	Red	K788-100-2A
GOx Substrate	1 ml	Blue	K788-100-3
GOx Developer	1 vial	Green	K788-100-4
GOx Positive Control	1 vial	Purple	K788-100-5
H ₂ O ₂ Standard (0.88 M)	0.1 ml	Yellow	K788-100-6

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 GOx 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:

- H₂O₂ Standard Curve:** Add 10 µl 0.88 M H₂O₂ Standard to 870 µl dH₂O to make 10 mM H₂O₂ Standard. Dilute 10 mM H₂O₂ Standard further to 1:9 with GOx Assay Buffer to make 1 mM H₂O₂ Standard. Add 0, 2, 4, 6, 8, 10 µl, of the diluted 1 mM H₂O₂ Standard into a series of wells of 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well H₂O₂ Standard. For the fluorometric assay, dilute 1 mM H₂O₂ Standard to 1:9 with GOx Assay Buffer to make 100 µM H₂O₂ Standard. Add 0, 2, 4, 6, 8, 10 µl of the diluted 100 µM H₂O₂ Standard into a series of wells of 96-well plate to generate 0, 0.2, 0.4, 0.6, 0.8, 1 nmol/well H₂O₂ Standard. Adjust the final volume to 50 µl with GOx Assay Buffer.
- Sample Preparation:** Homogenize cells (1×10⁶) 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.
- Positive Control:** Add 2-10 µl of Positive Control into the desired well(s) & adjust final volume to 50 µl with Assay Buffer.
- 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.

- Measurement:** Incubate the plate for 5 min at 37°C & measure OD at 570 nm or fluorescence at Ex/Em = 535/585 nm (A₁). Incubate for another 15 minutes to 2 hrs at 37°C & 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 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 min, dilute the sample and rerun the assay. The H₂O₂ Standard curve can read in end point mode (i.e. at the end of incubation time).
- Calculation:** Subtract the 0 Standard reading from all readings. Plot H₂O₂ Standard Curve. Calculate the glucose oxidase activity of the test sample: ΔOD = A₂ – A₁. Apply the ΔOD to the H₂O₂ Standard Curve to get B nmol of H₂O₂ generated by Glucose Oxidase during the reaction time (ΔT = T₂ – T₁).

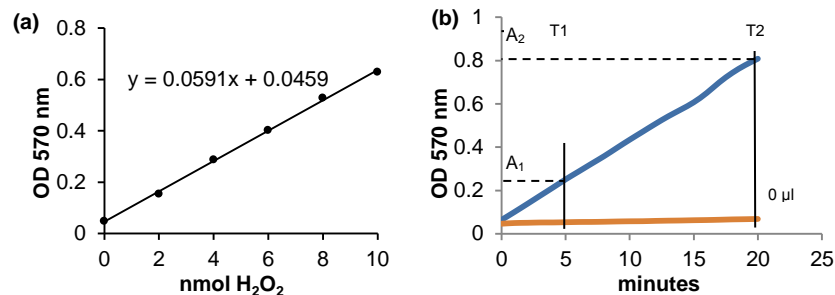
$$\text{Glucose Oxidase Activity} = \frac{B}{\Delta T \times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/ml}$$

Where: **B** is the H₂O₂ amount from the 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 H₂O₂ per min at 37°C.



Figures: H₂O₂ Standard Curve (a). Glucose oxidase activity in sample (b). Assays were performed following kit protocol.

VI. Related Products:

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| NAD/NADH Quantification Kit | NADP/NADPH Quantification Kit |
| ADP/ATP Ratio Assay Kit | Ascorbic Acid Quantification Kit |
| Glucose Assay Kit | Fatty Acid Assay Kit |
| Ethanol Assay Kit | Uric Acid Assay Kit |
| Pyruvate Assay Kit | Lactate Assay Kit // |
| Creatine Assay Kit | Creatinine Assay Kit |
| Ammonia Assay Kit | Free Glycerol Assay Kit |
| Triglyceride Assay Kit | Hemin Assay Kit |
| Choline/Acetylcholine Quantification Kit | Total Antioxidant Capacity (TAC) Assay Kit |
| Sarcosine Assay Kit | L-amino Acid Assay Kit |
| Nitric Oxide Assay Kit | Glutamate Assay Kit |

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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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.

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