Tribioscience

Fast Glucose Oxidase Activity Assay (Colorimetric/ Fluorometric)

One-step Sensitive Quantitation for Glucose Oxidase Activity

Catalog Number	Kit Size
TBS2088-200	200 assays
TBS2088-600	600 assays

DESCRIPTION

Glucose oxidase (GOD) 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.

Tribioscience's Fast Glucose Oxidase Activity Assay 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 570nm or fluorescence intensity at λex/em = 530/585 nm is directly proportional to glucose oxidase activity in the sample. The fluorometric assay is more sensitive than the colorimetric assay.

APPLICATIONS

Direct Assays: Glucose oxidase activity in serum, plasma, urine, and other bio-samples.

KEY FEATURES

Flexible: Suitable for colorimetric and fluorometric methods.

Accurate: Use 50 µL samples. Detection ranges 0.1-50 µM in 96-well plate for colorimetric assay and 0.002-10 µM for fluorometric assay.

Simple and H igh-Throughput: One-step procedure. Load-Incubate-Read. Kit can be used for a robust method.

Time Saving: Takes less than 30 minutes

KIT CONTENTS

Component	TBS2088-200	TBS2088-600
Assay Buffer	1x 12mL	3x12 mL
Red Probe	1x 0.25mL	1x 0.8 mL
GOD Standard Stock (1U/mL)	1x 25µL	1x 80µL
Enzymes	1x 2mL	3x 2mL

STORAGE AND HANDLING

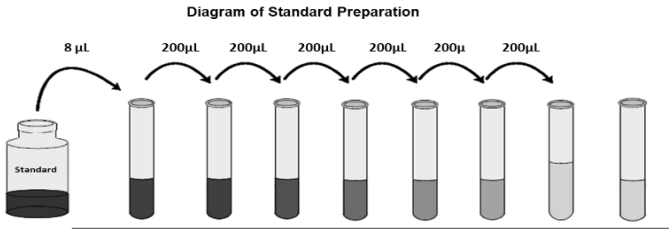
Store kit at -20°C. Shelf life of six months. Protect from light.

FLOROMETRIC PROTOCOL

Ensure the Reagent is at room temperature before use. Keep samples and enzyme on ice before the assay. It is recommended that all standards and samples be duplicated in the assay.

Sample Preparations:

Serum, Plasma, other body fluid, or cell culture supernatant can be measured directly by a series of dilutions of the sample (½, ¼, or ⅛). Solid samples such as tissue require processing. They can be homogenized and extracted with ethanol (80%) with a tissues/Ethanol ratio of 1:8 (1 hr at 4°C) followed by centrifugation at 10,000g. The Clear supernatants can then be measured as described for liquid samples. Add 10µL test samples directly into 96-well clear plate.



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	**392**	200	200	200	200	200	200	200
Stock (1 U/mL)	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Higher Std (µL)	**8**	200	200	200	200	200	200	
Final Conc (mU/mL)	20	10	5	2.5	1.25	0.625	0.3125	0

Standard Curve Preparations:

1. Label 1.5mL tubes from Std 1-8. As shown in the diagram.
2. Add 392µL of 1x Assay Buffer to Std1 and 200µL to Std 2-8.
3. Add 8 µL of 1.0 U/mL Glucose Oxidase stock solution to Std1, then add 200uL of Std1 to Std2. Carry out a 2x serial dilution for Std 3-7. Leave Std8 as pure 1x Assay Buffer to be standard 0. The standard concentration range is 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0 mU/mL (Note the final glucose oxidase concentration will be two fold lower).

Work Solution

Mix 39µL Assay Buffer with 10µL Enzymes, and 1µL probe for 50uL per reaction.

Assay Procedures

1. Add 50µL of standard or sample to each well of a black microplate in duplicate manner *(Note: the black microplate is for fluorescence detection)*.
2. Add 50µL work solution to each well containing the Standard and test samples. Tap plate lightly to mix.
3. Incubate at room temperature for 30 minutes protected from light.
4. Measure Fluorescence value at 570nm (565-585 nm) in plate reader. Measure the fluorescence using a microplate reader, equipped for excitation in the range of 530-560nm and emission detection at ~ 590nm. *(Note: Because the assay is continuous (not terminated), fluorescence or absorbance may be measured at multiple time points to follow the kinetics of the reactions).*

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COLORIMETRIC PROCEDURE

The colorimetric assay is similar to the fluorometric assay. But its sensitivity is much lower than the fluorometric assay. The linear detection range is 0.1 to 50 mU/mL glucose oxidase. Prepare the standards using the fluorometric procedure to obtain standards at 20, 10, 5, 2.5 1.25, 0.625 0.3125 and 0 µM.

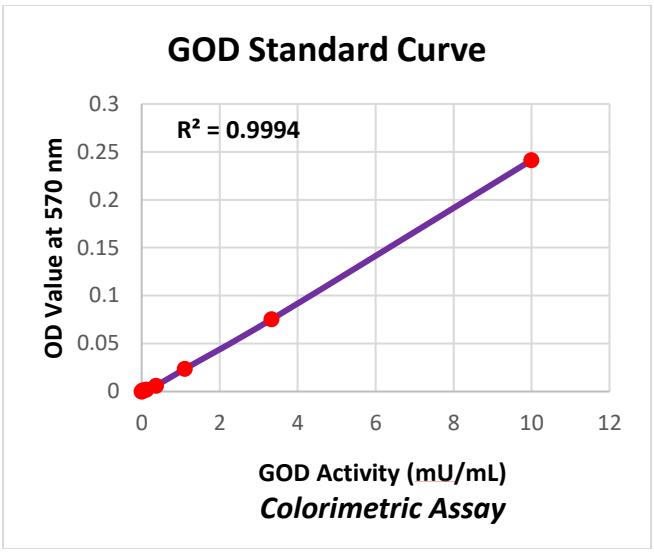
1. Transfer 50µL standards, samples into separate wells of a 96-well plate.
2. Add 50µL Working Reagent (see fluorometric Procedure), tap plate to mix. Incubate 30 min at room temperature.
3. Read OD value at 570 nm (550-585 nm).

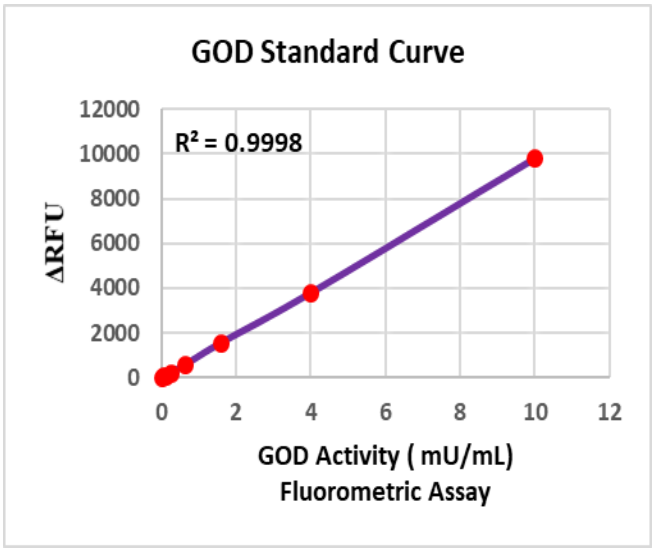
Calculation

Subtract the blank value (0 µM Standard) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the glucose concentration of the Sample using the equation obtained from the linear regression of the standard curve.

Glucose = N x (R_{sample}- R_{blank})/Slope (µM)

Where: Rsample and Rblank are optical density or fluorescence intensity readings of the sample and blank, respectively. N is the sample dilution factor.





RELATED PRODUCTS

Cell Viability Assay Kits (Catalog# TBS2001)
ATP Colorimetric/Fluorometric Assay (Catalog# TBS2010)
ADP Colorimetric/Fluorometric Assay Kit (Catalog# TBS2020)
Glucose Oxidase Colorimetric/Fluorometric Assay (Catalog# TBS2087)

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