Tribioscience

Fast Glucose Determination Assay (Colorimetric/Fluorometric)

One-step Sensitive Quantitation for Glucose Determination

Catalog Number	Kit Size
TBS2087-200	200 assays
TBS2087-600	600 assays

DESCRIPTION

Glucose is the most important carbohydrate in biology. It is a primary source of energy for the body's cells transported through the blood stream. As such, glucose levels need to be highly regulated in the human body. Failure to regulate blood glucose within the normal range leads to conditions of persistently high or low blood sugar. Diabetes mellitus is the most prominent disease related to improper blood sugar regulation. The determination of glucose levels in blood is critical in the control of diabetes.

Fast Glucose Determination Assay (Colorimetric/Fluorometric) provides a rapid, simple, reproducible, and sensitive approach for measuring glucose in plasma, serum, urine, and other bio-samples. The glucose assay uses the glucose oxidase-peroxide reaction to measure glucose concentrations. The color intensity of the reaction product at 570nm or fluorescence intensity at λem/ex = 585/530 nm is directly proportional to the glucose concentration in the sample.

APPLICATIONS

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

KEY FEATURES

Flexible: Suitable for colorimetric and fluorometric methods.
Accurate: Use 10µL samples. Detection ranges 0.4-200 µM in 96-well plate for colorimetric assay and 1-50 µM for fluorometric assay.
Simple and High-Throughput: One-step procedure: just load-incubate-read. Kit can be used for a robust method.
Time Saving: Takes less than 30 minutes

KIT CONTENTS for 100 Assays

Component	TBS2087-200	TBS2087-600
5x Assay Buffer	1x 12mL	3x 12mL
Red Probe	1x 0.25mL	1x 0.8mL
Glucose Standard Stock (2mM)	1x 100µL	1x 300µL
Enzymes	1x 2mL	3x 2mL

STORAGE AND HANDLING

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

FLUOROMETRIC PROTOCOL

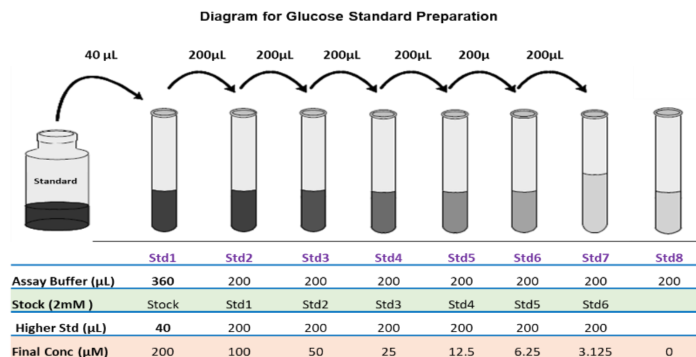
Ensure the Reagent is at room temperature before use. Keep samples and enzyme on ice before the assay. It is recommended that all standards and samples be duplicated in the assay.

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). For solid samples such as tissue, homogenize and extracti 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 then can be measured as described for liquid samples. Add 10µL test samples directly into 96-well clear plate.

Standard Curve Preparations

1. Label 1.5mL Std tubes 1-8. As shown below in the diagram.
2. Add 360µL of 1x Assay Buffer to Std1 and 200µL to Std 2-8.
3. Add 40 µL of 2mM Glucose Standard Stock solution to Std1 and 200µL of Std1 to Std2. Carry out a 2x serial dilution for Std 3-7. Leave Std8 as pure 1x Assay Buffer to be the 0 standard. The standard concentration range is 200, 100, 50, 25, 12.5, 6.25, 3.125 µM, and *0 (Note that final glucose concentration will be twofold lower, e.g., 0 to 100 µM)*.



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	360	200	200	200	200	200	200	200
Stock (2mM)	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Higher Std (µL)	40	200	200	200	200	200	200	
Final Conc (µM)	200	100	50	25	12.5	6.25	3.125	0

Work solution

Mix 48µL 1x Assay Buffer with 1µL enzymes and 1µL probe for 50µL per well.

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 Standards and test samples. Tap plate lightly to mix.
3. Incubate at room temperature for 30 minutes protected from light.
4. Measure Fluorescence value at λ ex/em = 530/585 nm in a plater reader *(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 that of the fluorometric assay. 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 (0µM 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# TBS2088)

Research Use only.