Product Manual

Glucose Assay Kit (Colorimetric)

Catalog Number

STA-680 **500 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Glucose is a sugar used as an important source of energy in plants, prokaryotes and eukaryotes via processes such as respiration and fermentation. In plants, algae, and cyanobacteria, the energy of light is synthesized into the storage form of sugars such as glucose. More specifically, in a downstream process known as the Calvin cycle, carbon dioxide is incorporated into organic carbon compounds, like ribulose bisphosphate. Using ATP and NADPH from upstream light-dependent reactions, the resulting compounds are then reduced and removed to form further carbohydrates, such as glucose.

In animals, through the process of glycolysis followed by the citric acid cycle, glucose is broken down to water and CO_2, resulting in energy from ATP formation. Glucose is often stored as a polymer such as glycogen. In humans, glucose is commonly measured in blood samples. Bloodstream levels of glucose are normally under tight regulation; however, high levels measured in fasting individuals may indicate prediabetes or diabetes.

Cell Biolabs' Glucose Assay Kit is a simple colorimetric assay that measures the amount of total glucose present in foods or biological samples in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 500 assays, including blanks, glucose standards and unknown samples. Sample glucose concentrations are determined by comparison with a known glucose standard. The kit has a detection sensitivity limit of 6.25 µM glucose.

Assay Principle

Cell Biolabs' Total Glucose Assay Kit measures total glucose within food or biological samples. Glucose is oxidized by glucose oxidase into D-gluconic acid plus hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific colorimetric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples are compared to a known concentration of glucose standard within the 96-well microtiter plate format. Samples and standards are incubated for 30-45 minutes and then read with a standard 96-well colorimetric plate reader (Figure 1).

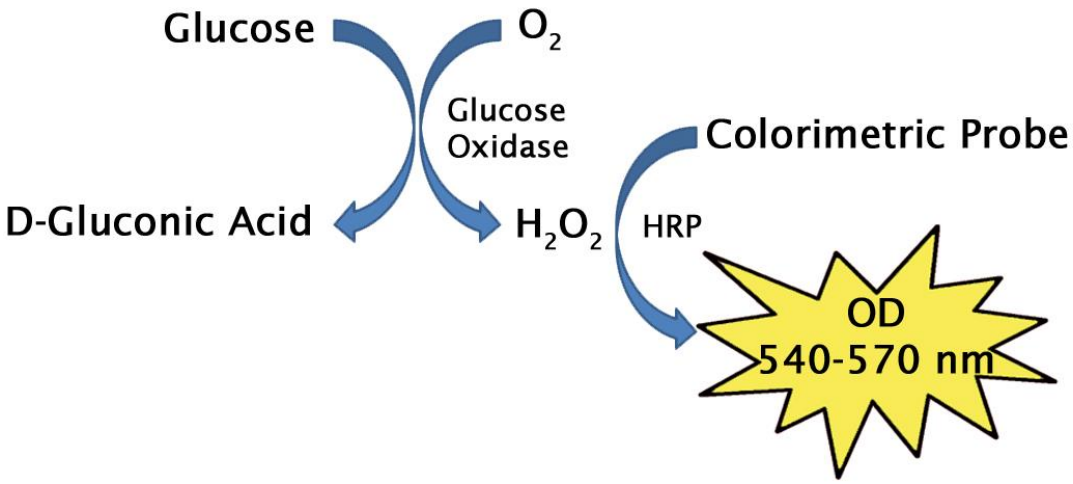

Figure 1. Glucose assay principle.

Related Products

1. STA-398: Free Glycerol Assay Kit (Colorimetric)
2. STA-672: S-Adenosylmethionine (SAM) ELISA Kit
3. STA-674: Glutamate Assay Kit
4. STA-675: Hydroxyproline Assay Kit
5. STA-682: Total Carbohydrate Assay Kit

Kit Components

1. Glucose Standard (Part No. 268001): One 500 µL tube at 400 mM
2. 10X Assay Buffer (Part No. 268002): One 25 mL bottle
3. Colorimetric Probe (Part No. 268003): One 250 µL amber tube
4. HRP (Part No. 234402): One 100 µL tube at 100 U/mL in glycerol
5. Glucose Oxidase (Part No. 268504): One 500 µL tube at 200 U/mL

   Note: One unit is defined as the amount of enzyme that will oxidize 1.0 micromole of beta-D-glucose to D-gluconic acid and hydrogen peroxide per minute at pH 5.1 at 35°C.

Materials Not Supplied

1. Distilled or deionized water
2. 1X PBS
3. Standard 96-well clear microtiter plate and/or clear cell culture microplate
4. Spectrophotometric microplate reader capable of reading in the 540-570 nm range.

Storage

Upon receipt, store the Glucose Standard, Colorimetric Probe, HRP, and Glucose Oxidase at -20ºC. The Colorimetric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles. Store the 10X Assay Buffer at room temperature.

Preparation of Reagents

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity.
- Reaction Mix: Prepare a Reaction Mix by diluting the Colorimetric Probe 1:100, HRP 1:500, and Glucose Oxidase 1:50 in 1X Assay Buffer. For example, add 50 µL Colorimetric Probe stock solution, 10 µL HRP stock solution, and 100 µL of Glucose Oxidase to 4.84 mL of 1X Assay Buffer for a total of 5 mL. This Reaction Mix volume is enough for 100 assays. The Reaction Mix is stable for 1 day at 4ºC.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.

Preparation of Samples

- Cell culture supernatants: Cell culture media containing glucose should be avoided. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The cell conditioned media may be assayed directly or diluted as necessary. Prepare the Glucose standard curve in non-conditioned media without glucose.
  Note: Maintain pH between 7 and 8 for optimal working conditions as the Colorimetric Probe is unstable at high pH (>8.5).
- Tissue lysates: Sonicate or homogenize tissue sample in cold PBS or 1X Assay Buffer and centrifuge at 10000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in 1X Assay Buffer.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed directly or diluted as necessary in 1X Assay Buffer.

Notes:

- *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
- *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Colorimetric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).*
- *Avoid samples containing DTT or β-mercaptoethanol since the Colorimetric Probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh Glucose standards before use by diluting in 1X Assay Buffer. First, dilute the stock 400 mM Glucose Standard solution 1:10 in 1X Assay Buffer to yield a 40 mM Glucose Solution (e.g. add 5 µL of the stock 400 mM Glucose Standard to 45 µL of 1X Assay Buffer). Use the 40 mM Glucose Solution to prepare a series of the remaining Glucose standards according to Table 2 below.

Standard Tubes	40 mM Glucose Solution (µL)	1X Assay Buffer (µL)	Glucose (µM)	Glucose (mg/dL)
1	4	1596	100	1.8
2	250 of Tube #1	250	50	0.9
3	250 of Tube #2	250	25	0.45
4	250 of Tube #3	250	12.5	0.225
5	250 of Tube #4	250	6.25	0.113
6	250 of Tube #5	250	3.13	0.056
7	250 of Tube #6	250	1.56	0.028
8	0	250	0	0

Table 2. Preparation of Glucose Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 50 µL of each glucose standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 50 µL of Reaction Mix to each well. Mix the well contents thoroughly and incubate for 30-45 minutes at 37ºC protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
4. Read the plate with a spectrophotometric microplate reader in the 540-570 nm range.
5. Calculate the concentration of glucose within samples by comparing the sample OD to the standard curve.

Example of Results

The following figures demonstrate typical Glucose Assay Kit (Colorimetric) results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

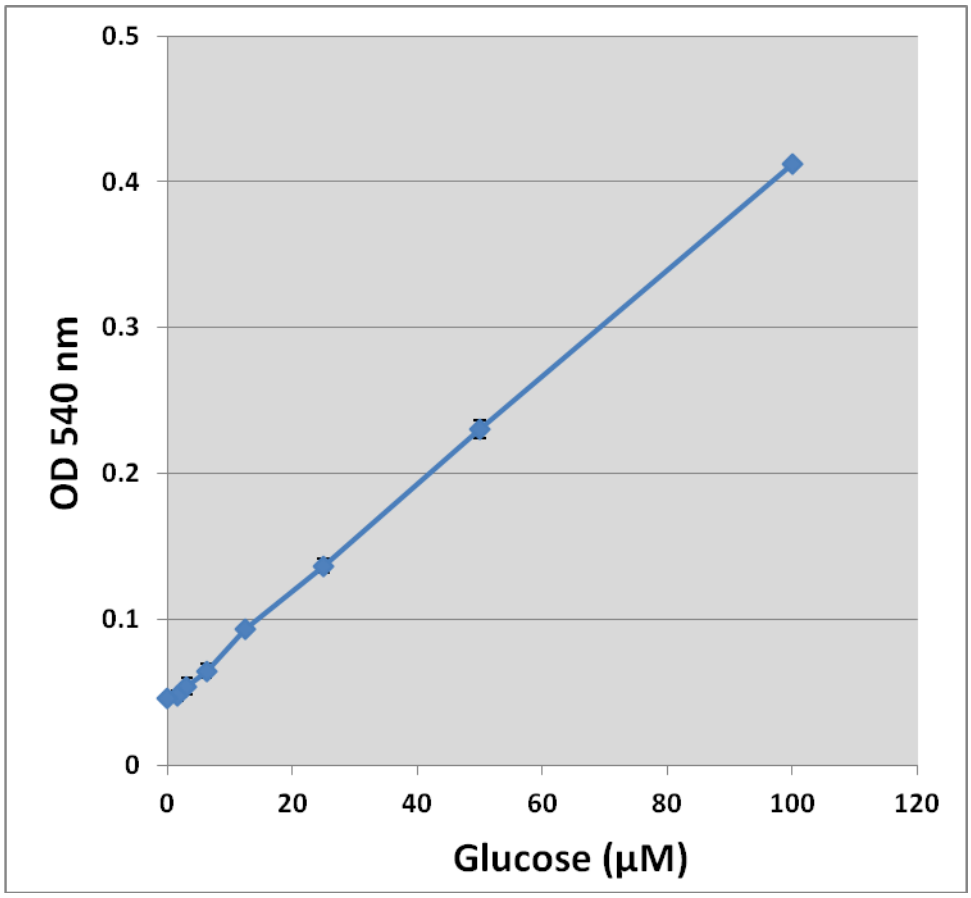

Figure 2: Glucose standard curve.

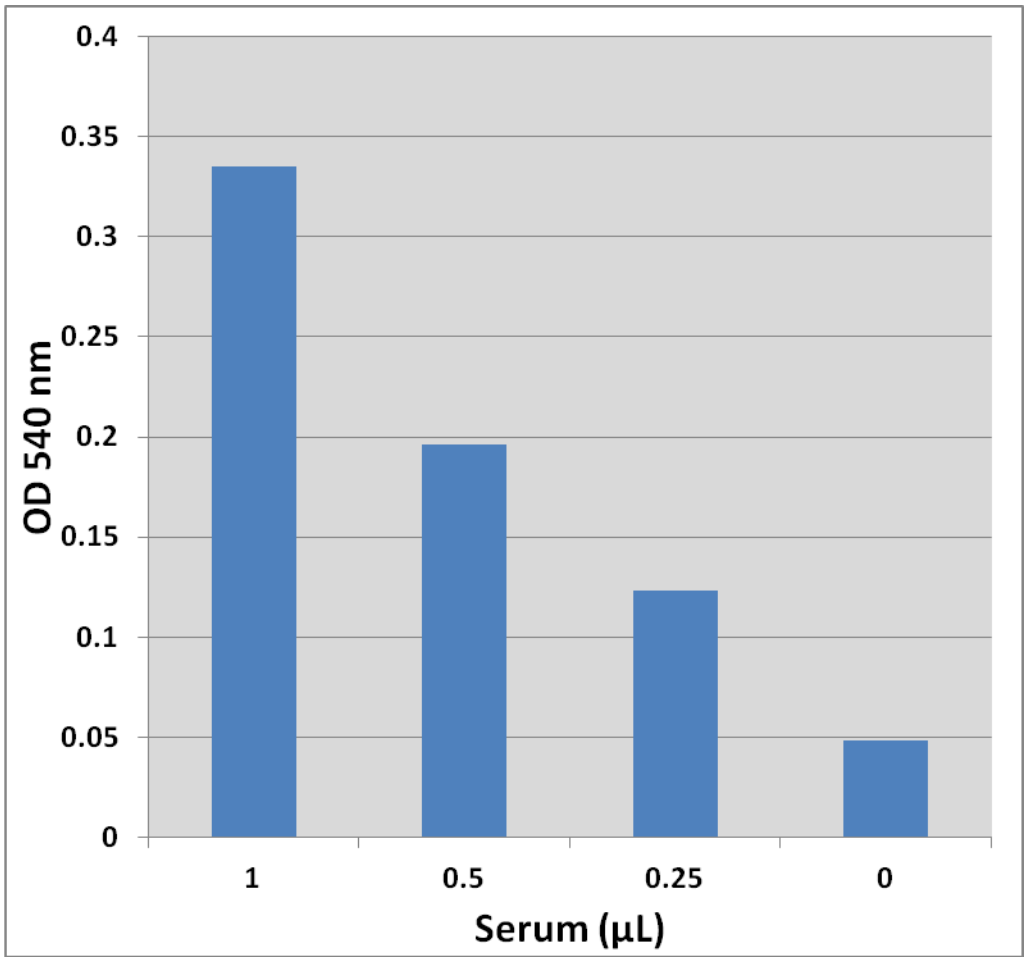

Figure 3: Glucose detection in human serum using the Glucose Assay Kit (Colorimetric).

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Recent Product Citations

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