Datasheet for ABIN5067616

Glucose Assay Kit (Fluorometric)

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Overview

Quantity:	500 tests
Reactivity:	Bacteria, Human
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	Total Glucose Assay Kit measures total glucose within food or biological samples.
Detection Method:	Fluorometric
Sensitivity:	1.56 µM
Characteristics:	Glucose Assay Kit is a simple fluorometric 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 1.56 µM glucose.
Components:	1. Glucose Standard : One 500 µL tube at 400 mM 2. 10X Assay Buffer : One 25 mL bottle 3. Fluorometric Probe : One 250 µL amber tube 4. HRP : One 100 µL tube at 100 U/mL 5. Glucose Oxidase : 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.

Target Details

Background:	Glucose is a sugar used as an important source of energy in plants, prokaryotes and eukaryotes

Target Details

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 CO2, 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 (Table 1), however, high levels measured in fasting individuals may indicate prediabetes or diabetes. Blood Glucose Range Animal (mg/dL) (mM) Human 79-110 4.4-6.1 Cow 42-75 3.7-6.6 Sheep 44-81 3.9-7.2 Goat 48-76 4.2-6.7 Cat 61-124 5.4-11.0 Dog 62-108 5.5-9.5 Horse 62-114 5.5-10.1 Pig 66-116 5.8-10.3 Rabbit 75-155 6.6-13.7 Llama 90-140 8.0-12.4 Mountain Goat 26-181 2.3-16.0 Beluga Whale 94-115 8.3-10.2 White Rhinoceros 28-140 2.5-12.4 Harp Seal 88-218 7.8-19.3 Hooded Seal 135-283 11.9-25.0 Table 1. Range of blood glucose levels in common animals (Ref. 1).

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Protocol:	Glucose is oxidized by glucose oxidase into D-gluconic acid plus hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific fluorometric 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 45 minutes and then read with a standard 96-well fluorometric plate reader .
Reagent Preparation:	• 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 Fluorometric Probe 1:100, HRP 1:500, and Glucose Oxidase 1:50 in 1X Assay Buffer. For example, add 50 µL Fluorometric Probe stock solution, 10 µL HRP stock solution, and 100 µL of Glucose Oxidase to 4.84 mL 1X Assay Buffer for a total of 5 mL. The above example Reaction Mix volume is enough for 100 assays. The Reaction Mix is stable for 1 day at 4 °C. Note: Prepare only enough for immediate use by scaling the above example proportionally. 4
Sample Preparation:	• Cell culture supernatants: Cell culture media formulated with glucose should be avoided. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The cell conditioned media

Application Details

	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 Fluorometric 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 106 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. Dilute the supernatant from 1:50 to 1:200 with 1X Assay Buffer just prior to performing the assay. 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 Fluorometric 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 Resorufin is not stable in the presense of thiols (above 10 µM).
Assay Procedure:	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 black microtiter plate suitable for a fluorescence plate reader. 3. Add 50 µL of Reaction Mix to each well. Mix the well contents thoroughly and incubate for 30-45 minutes at 37 °C 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 fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range. 5. Calculate the concentration of glucose within samples by comparing the sample RFU to the standard curve.
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	RT/-20 °C
Storage Comment:	Upon receipt, store the Glucose Standard, Fluorometric Probe, HRP, and Glucose Oxidase at -20°C. The Fluorometric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles. Store the 10X Assay Buffer at room temperature.

Publications

Product cited in: Kuang, Jahangiri, Mascharak, Nguyen, Chandra, Flanigan, Yagnik, Wagner, De Lay, Carrera, Castro, Hayes, Sidorov, Garcia, Eriksson, Ronen, Phillips, Molinaro, Koliwad, Aghi: "GLUT3 upregulation promotes metabolic reprogramming associated with antiangiogenic therapy resistance." in: **JCI insight**, Vol. 2, Issue 2, pp. e88815, (2017) (PubMed).

Images

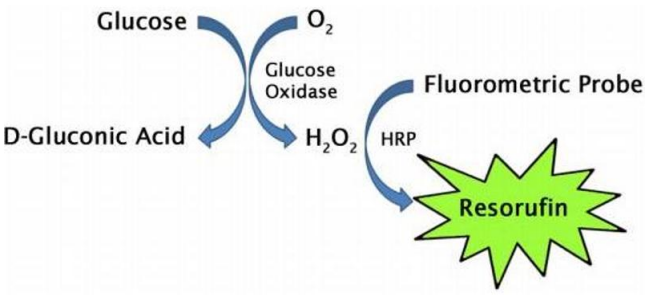
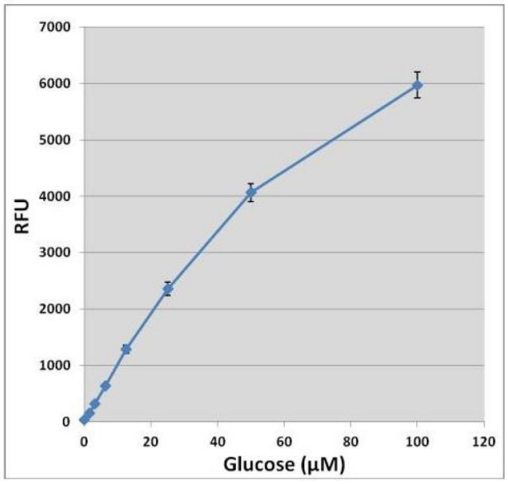
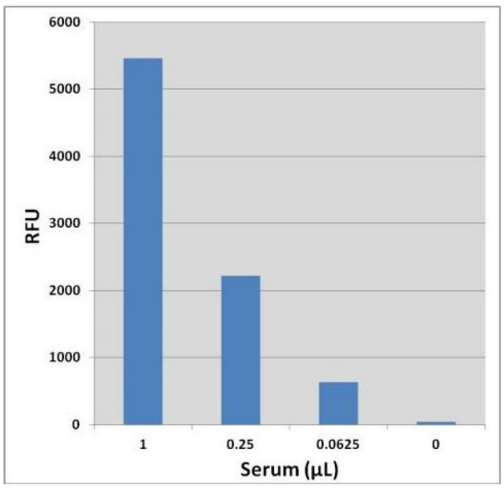


Image 1. Glucose assay principle.



Biochemical Assay

Image 2. Glucose standard curve.



Biochemical Assay

Image 3. Glucose detection in human serum using the Glucose Assay Kit (Fluorometric).