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IDTox™ Glucose Assay Kit

SUP6016

Enzyme Immunoassay for the determination of the glucose in serum samples.

PRODUCT DESCRIPTION

The ID Labs is a plate-based colorimetric enzymatic assay for the determination of glucose in serum samples. Glucose is the most prominent carbohydrate found in blood. The metabolism of glucose is the chief source of cellular energy throughout the body. The determination of serum glucose is an important marker for a number of disease states. Elevated levels of glucose are strongly associated with diabetes and other diseases such as liver or thyroid diseases, renal failure, and pancreatitis while low glucose levels are linked to insulin-induced hypoglycemia and neoplasms.

The kit uses a spectrophotometric, kinetic assay to detect glucose directly from serum samples. The features of the kit are:

- High sensitivity and low detection limit (20 U/L)
- A rapid (5 minutes) and robust enzyme-based assay which does not require expensive instrumentation
- High reproducibility
- Only 5 ml of Serum needed per well.

PROCEDURE OVERVIEW

The Glucose Assay Kit is a simple, direct and automation-compatible method for measuring glucose levels in serum samples. This kit uses a coupled enzymatic reaction scheme: Glucose is first oxidized by glucose oxidase enzyme to form gluconic acid and hydrogen peroxide. In the second step, the hydrogen peroxide reacts with a chromogenic dye using peroxidase to form a visibly-colored (red) dye product. The amount of dye product, measured by the sample absorbance at 520 nm, is proportional to the concentration of glucose in the sample. The kit comes with a control solution containing a glucose standard (100 mg/dl), which is used to calibrate the assay.

KIT REAGENTS SUPPLIED

The Glucose Assay Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for standards). The kit also contains enough material to construct four standard curves. Store the kit at 4°C, except for the plate, which is stored at RT. The shelf life of the kit, when properly stored is noted on the label. Once the Reagent Mix is reconstituted the shelf life of the mix is 2 months when properly stored. For more details, see “Preparation of Reagent Mix”

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	RT
Reagent Mix	Vial	2-8ºC
Glucose Standard (100 mg / dl)	900 µl	2-8ºC

MATERIALS REQUIRED BUT NOT PROVIDED

Microtiter plate reader (520 nm).
PBS, pH 7.3
Centrifuge to prepare serum samples.
Deionized or distilled water.
1.5 ml microfuge tubes.
Multichannel pipette or repeating pipettor (*recommended but not required*).

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WARNINGS AND PRECAUTIONS

It is strongly recommended that you read the following warnings and precautions to ensure your full awareness of the techniques and other details you should pay close attention to when running the assays. Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the protocol coming with the kit.
Add standards to plate only in the order from low concentration to high concentration, as this will minimize the risk of compromising the standard curve. Try to maintain a laboratory temperature of (20–25°C/68–77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation. Be sure to use only distilled or deionized water since water quality is very important.
When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic. Wear gloves when performing the procedure. Add standards to plate only in the order from low concentration to high concentration, as this will minimize the risk of compromising the standard curve.

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ASSAY PROCEDURE

PREPARATION OF REAGENTS
Serum
1. Carefully collect whole blood in a 1.5 ml microfuge tube or serum collection tube making sure to avoid hemolysis as it may release erythrocyte glucose into the serum.
2. Incubate the blood sample at 37°C for 10 minutes.
3. Centrifuge sample at 10,000 rpm for 10 minutes.
4. Remove serum layer to a clean tube avoiding the “buffy coat” layer.
5. Store serum samples on ice or at 4°C prior to testing. Glucose in serum is stable for 1 - 2 days at 4°C and three months when frozen.

NOTE:
Samples with values above 300 mg/dl should be diluted 1:1 with PBS and re-tested. Multiply the results by two.
Grossly lipemic serums require a sample blank. Add 5 µl of sample to 300 µl PBS, mix and read the absorbance against water. Subtract this reading value from the absorbance of each serum sample to obtain the corrected reading

ASSAY PROCEDURE
Set up
Allow all reagents and the microtitre plate to warm up to room temperature before use.
If a temperature controlled plate reader is available, adjust the plate reader temperature control to 37°C and equilibrate the Master Mix at 37°C to obtain even higher sensitivity measurements.

Preparation of Reagent Mix
To reconstitute the Reagent Mix, add exactly 30 ml of deionized or distilled water to the Reagent Mix powder. Mix by swirling or inverting the bottle 10 - 12 times. Allow contents to dissolve for 10 minutes at room temperature.

IMPORTANT: The reconstituted Reagent Mix can be left at room temperature for short periods (30 – 60 minutes) prior to use. Between uses, the reconstituted Reagent Mix should be stored at 4°C (for up to 2 months). Discard the Reagent Mix 2 months after reconstitution.

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Preparation of Glucose Standard for Standard Curve

Label 6 microfuge tubes: 1, 2, 3, 4, 5, 6 Neg. Then make 6 dilutions of the Glucose Standard using distilled or deionized water (dH2O) as described in the table below.

NOTE: There is enough material to construct 3 Standard Curves. Make the Glucose Standard dilutions for the Standard Curve fresh each time that the Standard Curve is performed. After each dilution, briefly mix the tube before performing the next dilution.

Std Tube #	Preparation	Glucose (mg/dl)
1	Add 100 µl of Glucose Standard.	100
2	Add 80 µl of Glucose Standard 1 + 20 µl dH_2O. Mix thoroughly.	80
3	Add 60 µl of Glucose Standard 1 + 40 µl dH_2O. Mix thoroughly.	60
4	Add 40 µl of Glucose Standard 1 + 60 µl dH_2O. Mix thoroughly.	40
5	Add 20 µl of Glucose Standard 1 + 80 µl dH_2O. Mix thoroughly.	20
6 (Neg)	Add 100 µl of dH_2O.	0

Test Procedure

1. Add 5 µl of each sample or standard (in duplicate) to the microplate wells.
2. Add 300 µl of Reagent Mix to the wells.
3. Incubate at 37°C for 10 minutes or room temperature for 20 minutes.
4. Measure the absorbance of each sample at 520 nm.

CALCULATION OF RESULTS

Glucose Concentration Calculation

There is a linear relationship between the concentration of glucose in the sample and absorbance at 520 nm. Therefore, a standard curve used to calculate the glucose concentration in serum samples can be constructed by plotting the mean corrected absorbance values for each of the diluted glucose standards as a function of glucose concentration (mg/dl).

The straight line which best fits the data of Standard Curve can be used to calculate the glucose concentration in each sample.