Catalog# 81692

V.3

Mouse Glucose Assay Kit Instructions

For the quantitative determination of glucose
in mouse serum, plasma, and urine

Catalog# 81692
Or #81694 (10-Kit Pack)

96 Assays

For research use only. Not for use in diagnostic procedures.

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A. Intended Use

The Mouse Glucose Assay kit is for the quantitative determination of glucose in mouse serum, plasma, and urine. The glucose concentration is expressed as mg/dL. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Glucose is a small organic compound with the molecular formula $C_6H_{12}O_6$. The body breaks down many carbohydrates in food to form glucose which is the major source of energy used in the body. Levels of glucose are controlled by a variety of hormones, and levels can also fluctuate based on diet, exercise, and a range of diseases. Monitoring glucose levels is essential to diabetes research.

C. Principle of the Assay

The Mouse Glucose Assay kit is based on a multi-step reaction. To start, α-D-glucose is rapidly converted to β-D-glucose in the presence of mutarotase. The β-D-glucose is oxidized and yields hydrogen peroxide as a by-product. In a closing reaction, the hydrogen peroxide reacts with chemical reagents to yield a red dye. The dye formation is monitored by measuring absorbance at 505nm and is directly proportional to the glucose concentration in the mouse sample.

D. Kit Storage

1. Upon receipt of the Mouse Glucose Assay kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
BUF	Buffer	2 x 15 mL
CC1	Reagent 1 (lyophilized)	2 x 1 vial
CAL	Calibrator (Liquid, 500 mg/dL)	1 mL

E.2. Materials required but not provided

Microplate or glass test tubes
Micropipettes and disposable tips
Polypropylene microtubes
Incubator (37ºC)
Distilled or deionized water
Microplate reader or spectrophotometer (able to read A_{505} values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components may contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.

G. Maximizing Kit Performance

1. Given the small sample volumes required (2 µL), pipetting should be done as carefully as possible. A high quality 10 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each standard and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Fresh serum or plasma samples should be used and can be stored for 1 day at room temperature or 3 days at 2-8°C. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Since glucose levels decrease in whole blood upon storage, blood cells should be separated as soon as possible. Samples collected with heparin, citrate, oxalate, EDTA and sodium fluoride are compatible with this test when used in typical amounts. Excessive hemolysis should be avoided.

I. Assay Procedure

I.1. Preparation of reagents

1. Buffer
   Provided as ready to use.
2. Reagent 1
   Reconstitute the reagent with 15 mL of the buffer by pouring one of the bottles of buffer into one vial of Reagent 1. Mix well. The reconstituted reagent is stable for 1 month when stored at 2-8°C. If running the entire kit at one time, pool both reconstituted portions of Reagent 1 together before using.
3. Calibrator
   The calibrator concentration is 500 mg/dL and can be diluted with distilled or deionized water to prepare working standards as described in **I.2**.

I.2. Preparation of standards

1. Pipette 50 µL of distilled or dionized water into four polypropylene microtubes labeled 250, 125, 62.50, and 31.25 mg/dL.
2. Dispense 50 µL of the 500 mg/dL calibrator into the 250 mg/dL microtube, and mix thoroughly.
3. Dispense 50 µL of the 250 mg/dL standard into the 125 mg/dL microtube, and mix thoroughly.
4. Dispense 50 µL of the 125 mg/dL standard into the 62.5 mg/dL microtube, and mix thoroughly.
5. Dispense 50 µL of the 62.5 mg/dL standard into the 31.25 mg/dL microtube, and mix thoroughly.
6. Dispense 50 µL of distilled or deionized water into one polypropylene microtube labeled 0 mg/dL.
7. Dispense 50 µL of the 500 mg/dL calibrator into a microtube labeled 500 mg/dL.

You now have working standards of 500, 250, 125, 62.5, 31.25, and 0 mg/dL.

I.3. Assay procedure

The procedure below reflects a manual procedure performed using a microplate and a microplate reader (ideal when running multiple samples manually). The procedure can be easily adopted as needed to be run in a glass tube with a spectrophotometer.

1. Add 300 µL of the reconstituted Reagent 1 into the wells to be used.
2. Add 2 µL of sample or working standard into the appropriate wells, and mix by repeated pipetting.
3. Incubate the microplate at 37ºC for 5 mins.*
4. Measure absorbance using a plate reader at 505nm within 15mins.
   **Alternatively, if an incubator is not available, the assay can be run at room temperature with an incubation time of 15mins.*

I.4. Determining the mouse glucose concentration

1. Using computer software or graph paper, construct the mouse glucose calibration curve by plotting the mean absorbance value for each calibrator (incl. blank) on the Y axis versus the corresponding glucose concentration on the X axis. A linear fit should be used.
   Note: *A calibration curve should be plotted every time the assay is performed.*
2. Mouse glucose concentrations in the samples are interpolated using the calibration curve and mean change in absorbance values for each sample. The glucose concentration is expressed as mg/dL.
   Note: *Samples with a high glucose concentration (675 mg/dL or higher) should be diluted with distilled or deionized water and rerun.*

I.5. Comparing blood glucose levels to serum or plasma glucose levels

Glucose levels are most commonly measured in blood, serum, and plasma samples. That said, glucose levels across these samples are not directly comparable, as glucose is soluble and is thus more concentrated in plasma and serum than blood samples on a per volume basis (ie. higher values in plasma/serum compared to whole blood). Given this discrepancy, the industry standard is moving to plasma glucose levels, given lab results are generally reported as such.

Most hand-held glucometers try to compensate for this variation by using a calibration factor to report plasma glucose levels relevant for human samples (typically 1.11).[1] However, mice have higher hematocrit values than humans, so on a per volume basis, plasma glucose values will be higher than reported by hand-held glucometers intended for human samples. As a result, the Crystal Chem glucose kit provides a more reliable method of measuring glucose levels in rodents regardless of sample type, as it directly measures glucose levels in the sample without the need for a conversion factor. It is generally expected that this kit will produce higher (and more accurate) values than those obtained from a glucometer or other methods intended for human samples.[2] Therefore, once a suitable sample type has been determined, all experiments should use the same sample type and method for the most consistent interpretation across different sets of data.

J. Performance characteristics

J.1. Assay range

The Mouse Glucose assay has a linear range from 0-675 mg/dL.

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

K. References

1. D'Orazio, P., et al. "Approved IFCC recommendation on reporting results for blood glucose." *Clin Chem Lab Med* 2006; 44(12): 1486-1490.
2. Ayala, J. E., et al. "Standard operating procedures for describing and performing metabolic tests of glucose homeostasis in mice." *Dis Model Mech* 2010; 3: 525-534.

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