Glucose Assay Kit

Glucose Assay Kit can be used to measure Glucose in Serum, plasma, cell culture supernatants, food and beverage.

Catalog number: ARG82281

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INTRODUCTION

Glucose is a simple sugar with the molecular formula C6H12O6. Glucose is the most abundant monosaccharide, a subcategory of carbohydrates. Glucose is mainly made by plants and most algae during photosynthesis from water and carbon dioxide, using energy from sunlight. There it is used to make cellulose in cell walls, which is the most abundant carbohydrate. In energy metabolism, glucose is the most important source of energy in all organisms. Glucose for metabolism is partially stored as a polymer, in plants mainly as starch and amylopectin and in animals as glycogen. Glucose circulates in the blood of animals as blood sugar. The naturally occurring form of glucose is d-glucose, while l-glucose is produced synthetically in comparatively small amounts and is of lesser importance. Glucose is a monosaccharide containing six carbon atoms and an aldehyde group and is therefore referred to as an aldohexose. The glucose molecule can exist in an open-chain (acyclic) and ring (cyclic) form, the latter being the result of an intramolecular reaction between the aldehyde C atom and the C-5 hydroxyl group to form an intramolecular hemiacetal. In water solution both forms are in equilibrium and at pH 7 the cyclic one is the predominant. Glucose is a primary source of energy for living organisms. It is naturally occurring and is found in fruits and other parts of plants in its free state. In animals glucose arises from the breakdown of glycogen in a process known as glycogenolysis. [Wikipedia: Glucose]

PRINCIPLE OF THE ASSAY

This Glucose Activity Assay Kit provides a Simple, direct and automation-ready procedures for measuring glucose concentrations find wide applications in research and drug discovery. This Glucose Activity Assay Kit is designed to use an improved *o*-toluidine method measure glucose directly in serum or plasma without any pretreatment. (The kit is designed for serum and plasma, but may also be used with cell culture supernatants, food and beverage samples.) It utilizes a specific color reaction with glucose. The absorbance at 630nm is directly proportional to glucose concentration in the sample. The concentration of Glucose in the sample is then determined by comparing the signals of samples to the standard.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Glucose Reagent	50 ml (Ready to use)	4°C to RT
Glucose Standard (300 mg/dL)	1 ml	-20°C

The kit is shipped on ice. Store Glucose Reagent at 4°C to RT and store the standard at -20°C. Shelf life of 12 months after receipt.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 630 nm.
- Flat bottomed 96-well microplate
- Pipettes and pipette tips
- Deionized or distilled water
- Boiling water bath or heat block.
- 1.5-mL centrifuge tubes
- Tube holder

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The reagent contains acetic acid. This assay is preferably carried out in a chemical fume hood.
- Briefly spin down the reagents before use.
- It is recommended that the standards and samples be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Collect serum and assay immediately or aliquot & store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants- Remove particulates by centrifugation for 10 min at 1500 x g at 4°C and aliquot & store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Note: Samples should be clear and free of particles or precipitates. Avoid using haemolytic, icteric or lipaemic samples.

REAGENT PREPARATION

- Standard:

  - Dilute 300 mg/dL Glucose Standard solutions with deionized water to 300 mg/dL, 200 mg/dL, 100 mg/dL and 50 mg/dL as following table, and use deionized water serves as zero standard (0 µM).

  The example of the dilution of standards

Standard No.	Standard Conc. mg/dL	Deionized water (µl)	300 mg/dL stock Standard (µl)
S1	300	0	150
S2	200	50	100
S3	100	100	50
S4	50	125	25
S0	0	150	0

  The diluted standards could be stored at -20°C for future use.

- Samples:

  - if the Sample OD is higher than the Standard OD at 300 mg/dL, dilute sample in water and repeat the assay and the concentration read from the standard curve must be further converted by the appropriate dilution factor.

  - To determine low glucose concentrations, use 50 µL of sample and standards (instead of 5 µL) per 500 µL Reagent.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use, each vial should be mixed thoroughly without foaming and briefly centrifuge tubes prior to use.

1. Add **5 µL** of each standard (S0-S4) and sample to 1.5 ml centrifuge tube.
2. Add **500 µL** of Glucose Reagent in each tube. Close the tubes tightly and mix.
3. Place the tubes in a tube holder and heat in a boiling water bath or heat block at **100°C for 8 min**.
4. Cool down in **cold water bath** for **4 min**.
5. Transfer **200 µL** of each reaction including standards and samples in duplicate into a clear bottom 96-well plate. Careful: avoid bubble formation.
6. Read the OD with a microplate reader at **620-650 nm** (peak absorbance at 630nm) immediately.

Summary:

	Assayed sample	Standard S0-S4
Sample	5 µl	
Standards		5 µl
Glucose Reagent	500 µl	500 µl
Add in separate centrifuge tubes. Place the tubes in a tube holder and heat at 100°C for 8 min.		
Cool down in cold water bath for 4 min		
Transfer 200 µL of reaction solution into each well in a 96-well plat. Read the OD at 630nm immediately		

CALCULATION OF RESULTS

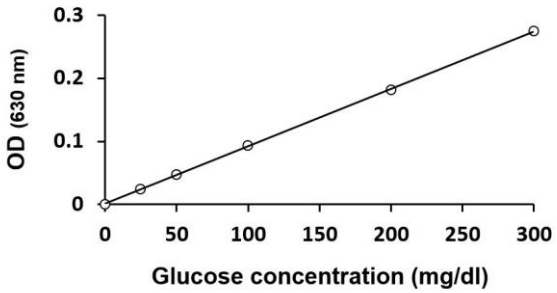
1. Subtract the blank value (S0) from the standard values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. and calculate the Glucose concentration of the Samples as follows:

   [Glucose] (mg/dL)=

   [(OD $_{SAMPLE}$ –OD $_{BLANK}$) / Slope]

2. The OD $_{Sample}$ and OD $_{Blank}$ OD are optical density values of the sample and S0 blank.
3. Typical serum/plasma glucose values: 70- 110 mg/dL.
4. Conversions: 1mg/dL glucose equals 55.5 µM, 0.001% or 10 ppm.
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor.

EXAMPLE OF ASSAY



EXAMPLE VALUES

The following results are example values determined by this assay kit. It is recommended that each laboratory establish its own normal range since corticosterone levels can vary due to handling and sampling techniques.

Sample	n	Concentration (mg/dL)	Coefficient of variance
Rat plasma	4	128 ± 2	< 3
Rat serum	4	72.5 ± 0.8	< 3
Goat serum	4	78.6 ± 0.6	< 3
Human plasma	4	69.3 ± 0.7	< 3

QUALITY ASSURANCE

Sensitivity

Use as little as 5 µL samples. Linear detection range 0.7 mg/dL (38.85 µM) to 300 mg/dL (16.6 mM) glucose in 96-well plate.

The minimum detectable dose (MDD) of Glucose was: 0.7 mg/dL (38.85 µM)