Product Information

Glucose Fluorometric Assay Kit

I. Kit Contents:

Components	K2221-100 100 assays	Cap Color	Part Number
Glucose Assay Buffer	25 ml	WM	K2221-C-1
PicoProbe™ (in DMSO)	0.4 ml	Blue	K2221-C-2
Glucose Enzyme Mix (Lyophilized)	1 vial	Green	K2221-C-3
Glucose Substrate Mix (Lyophilized)	1 vial	Red	K2221-C-4
Glucose Standard (100 mM)	100 µl	Yellow	K2221-C-5

II. Introduction:

Glucose ($C_6H_{12}O_6$) is an important fuel source to produce energy molecule ATP in virtually all living organisms. Serum glucose level is a key diagnostic parameter for many metabolic disorders. Measurement of glucose level is very important in both drug discovery and research processes.

The Glucose Fluorometric Assay Kit provides an ultra-sensitive, simple, fast and convenient way for detection of glucose levels in various samples based on fluorometric method. In the assay, D-glucose is enzymatically oxidized to generate a product which reacts with a colorless probe to yield the fluorescence (Ex/Em = 535/587). The fluorescence is directly proportional to the amount of glucose. The assay is suited for high-throughput screening. The kit can detect less than 0.5 µM glucose in various biological samples.

III. Application:

Measurement of glucose in various tissues/cells.

Analysis of metabolism and cell signaling.

Mechanistic study of obesity and diabetes.

IV. Sample Type:

Serum, plasma & other body fluids.

Animal tissues: liver, muscle, heart etc.

Cell culture: adherent or suspension cells.

Growth media.

Food.

V. User Supplied Reagents and Equipment:

96-well plate with flat bottom. White plates are preferred for this assay.

Multi-well spectrophotometer (ELISA reader).

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

PicoProbe™: Ready to use as supplied. Warm to room temperature before use. Store at -20°C.

Glucose Enzyme Mix: Reconstitute with 220 µl Glucose Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Stable for 2 months at -20°C.

Glucose Substrate Mix: Dissolve with 220 µl dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Stable for 2 months at -20°C.

VIII. Glucose Assay Protocol:

1. Sample Preparation: Liquid samples can be measured directly. Tissue (10 mg) or cells (1 x 10^6) should be homogenized on ice with 100 µl ice cold Glucose Assay Buffer. Centrifuge at 12,000 rpm for 5 min. Collect the supernatant. Add 1 - 50 µl sample (1 - 10 µg) into a 96 well plate and adjust the volume to 50 µl with Glucose Assay Buffer.

Notes:

A. Protein and various enzymes in samples may interfere with the assay, we recommend deproteinizing the samples using either a perchloric acid/KOH protocol or by spin filtering through a 10 kD membrane.

B. For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.

C. NADH in samples will generate background. For samples having high NADH levels, a sample background control may be required.

2. Standard Curve Preparation: Dilute Glucose Standard to 1 mM by adding 10 µl of 100 mM Glucose Standard to 990 µl dH_2O, mix well. Dilute 1 mM Glucose Standard further to 10 µM (10 pmol/µl) by adding 10 µl of 1 mM Glucose Standard to 990 µl of dH_2O. Mix well. Add 0, 2, 4, 6, 8 & 10 µl of 10 µM Glucose Standard into series of wells in 96 well plate to generate 0, 20, 40, 60, 80 and 100 pmol/well of Glucose Standard. Adjust volume to 50 µl/well with Glucose Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays (samples and Standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix
Glucose Assay Buffer	45 µl	47 µl
PicoProbe™	1 µl	1 µl
Glucose Enzyme Mix	2 µl	---
Glucose Substrate Mix	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing the Standard & test samples. Mix well.

Note: For samples having high NADH levels, add 50 µl of Background Control Mix to sample background control well(s). Mix well.

4. Measurement: Incubate the reaction for 30 minutes at 37°C, protected from light. Measure fluorescence at Ex/Em = 535/587 nm in a micro plate reader.

5. Calculation: Subtract 0 Glucose Standard reading from all readings. Plot the Glucose Standard curve. If sample background control reading is significantly high, subtract the background control reading from sample reading. Apply the corrected sample reading to the Glucose Standard curve to get B pmol of Glucose in the sample wells.

Sample Glucose concentration = B/V x Dilution Factor = pmol/µl = nmol/ml or µM

Where: B = amount of glucose in the sample from Standard curve (pmol).

V = sample volume added in the reaction well (µl).

Glucose in sample can also be expressed in nmol/mg of sample.

Glucose molecular weight: 180.2 g/mol.

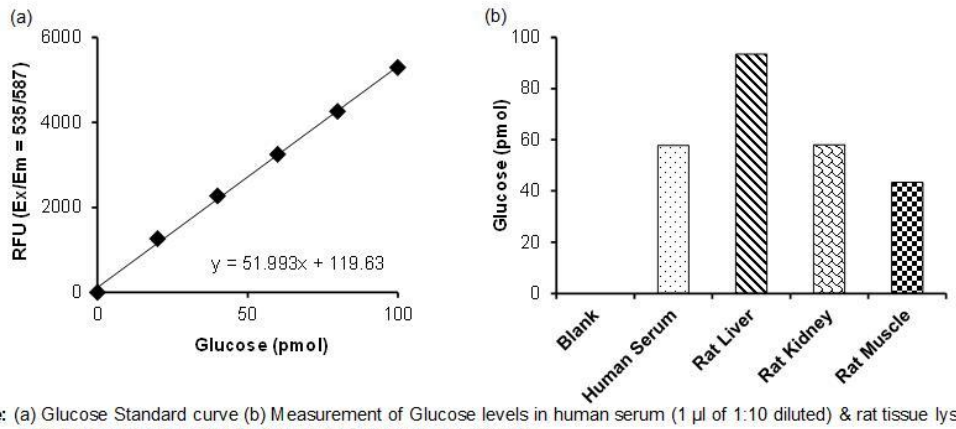

Figure: (a) Glucose Standard curve (b) Measurement of Glucose levels in human serum (1 µl of 1:10 diluted) & rat tissue lysates from liver, kidney & muscle (0.14 µg, 0.19 µg & 0.93 µg respectively).

Frequently Asked Questions

1. Can this kit be used with samples like bacteria, plants, drosophila etc?

We have optimized the kit with mammalian samples. However, theoretically these kits should work with samples from multiple species/sources. Since the optimal conditions depend on the sample type, the protocol has to be be adapted to fit the samples for efficient results. Please refer to this kits citations to see what kind of samples have been used with this kit other than mammalian samples.

2. Can we use frozen samples with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

3. Can we use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

4. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. You have to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

5. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

6. Why are my standard curve values lower than those shown on the datasheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, you can increase the incubation time. As long as the standard curve is linear, it should be fine to use, since all of your samples will also be measured under the same conditions on this curve.

7. How do I normalize my samples against protein concentration?

You can use a protein quantitation assay on the supernatants you get from cell/tissue lysates or with any other liquid sample in the assay buffer.

8. Can we use an alternate buffer for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

9. Should I make a standard curve for every expt I do, or is one curve/kit enough?

Yes, I would strongly recommend you to do the standards every time you do the expt. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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