Product Information

Glucose Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2091-100 100 assays	Cap Color	Part Number
Glucose Assay Buffer	25 ml	WM	K2091-C-1
Glucose Probe (in DMSO)	0.2 ml	Red	K2091-C-2
Glucose Enzyme Mix (lyophilized)	1 vial	Green	K2091-C-3
Glucose Standard (100 nmol/µ)	100 µl	Yellow	K2091-C-4

II. Introduction:

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

The Glucose Colorimetric/Fluorometric Assay Kit provides a sensitive, simple and convenient way for detection of glucose in various biological samples (serum, plasma, body fluid, growth medium, food, etc.) based on colorimetric and fluorometric method. In the assay, Glucose Enzyme Mix specifically oxidizes glucose to yield a product which reacts with a dye to produce fluorescence (Ex/Em = 535/587 nm) and color (λ = 570 nm). The color and fluorescence generated is proportionally to the amount of glucose. The assay is suited for high throughput screening and is also suited for monitoring glucose feeding in protein expression processes and glucose level during fermentation. The kit can detect 1-10000 µM glucose samples.

III. Application:

Measurement of Glucose in various samples.

Analysis of carbohydrate metabolism.

IV. Sample Type:

Serum, plasma, urine & other body fluids.

Growth media.

Food.

V. User Supplied Reagents and Equipment:

96-well plate with flat bottom.

Multi-well spectrophotometer.

VI. Storage and Handling:

Store kit at -20℃, protect from light. Briefly centrifuge small vials before opening. Read the entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

Glucose Assay Buffer: Warm to room temperature prior to use. Store at -20℃ or 4℃.

Glucose Probe: Ready to use as supplied. Warm to room temperature prior to use to melt frozen DMSO. Store at -20℃, protect from light and moisture. Use within two months.

Glucose Enzyme Mix: Dissolve in 220 µl Glucose Assay Buffer. Aliquot & store at -20℃. Keep on ice while in use. Use within two months.

VIII. Glucose Assay Protocol:

1. Sample Preparation: Add 2-50 µl test samples to a 96-well plate. Adjust the volume to 50 µl/well with Glucose Assay Buffer. If using serum, limit sample volume to 0.5-2 µl/assay. Normal serum contains ~5 nmol/µl glucose. Urine can be assayed directly. Adjust the final volume to 50 µl with Assay buffer.

Notes:

a. For unknown samples, we suggest performing a pilot experiment & testing different sample dilutions with the Assay Buffer to ensure the readings are within the Standard Curve range.

b. For samples having background, prepare parallel well(s) containing same amount of sample as in the test well.

c. Endogenous compounds may interfere with the reaction. To ensure accurate determination of Glucose in the test samples, we recommend spiking samples with a known amount of Standard (4 nmol).

d. Endogenous enzyme activity may cause loss of glucose. All samples containing enzyme activity should be deproteinized using a 10kDa Spin Column.

2. Standard Curve Preparation: For colorimetric assay, dilute the Glucose Standard to 1 nmol/µl by adding 10 µl of the Glucose Standard to 990 µl of Glucose Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells on a 96 well plate. Adjust volume to 50 µl/well with Glucose Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Glucose Standard. For the fluorometric assay, dilute the Glucose Standard solution to 0.1 nmol/µl by adding 10 µl of the Glucose Standard to 990 µl of Glucose Assay Buffer, mix well. Then take 20 µl into180 µl of Glucose Assay Buffer. Mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells as in the colorimetric assay. Adjust volume to 50 µl/well with Glucose Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the Glucose Standard.

3. Glucose Reaction Mix: Mix enough reagent for the number of assays to be performed: For each well, prepare a total 50 µl Reaction Mix containing

	Reaction Mix	Background Control Mix
Glucose Assay Buffer	46 µl	48 µl
Glucose Probe	2 µl	2 µl
Glucose Enzyme Mix	2 µl	--

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

Note:

For samples having background, add 50 µl of the background control mix to sample background control well(s)

The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Use 0.4 µl of the probe per reaction to decrease background/increase detection sensitivity significantly.

4. Measurement: Incubate the reaction for 30 min. at 37℃, protect from light. Measure absorbance (OD 570 nm) or Fluorescence (Ex/Em = 535/590 nm) for in a microplate reader.

5. Calculations: Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract the sample background control reading from sample reading. Plot the Glucose Standard Curve. For unspiked samples, apply the corrected absorbance or fluorescence to the Glucose Standard Curve to get B nmol of Glucose in the sample well.

Sample Glucose concentration (C) = B/V X D nmol/µl or mM.

Where: B is the amount of Glucose in the sample well (nmol).

V is the sample volume added into the reaction well (µl).

D is the sample dilution factor.

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, Glucose amount in sample well (B) = (OD sample (corrected))/ (OD $_{\text{sample + Chol Std(corrected)}}$× OD $_{\text{sample (corrected)}}$))* Glucose Spike (nmol)

Glucose molecular weight: 180.2 g/mol.

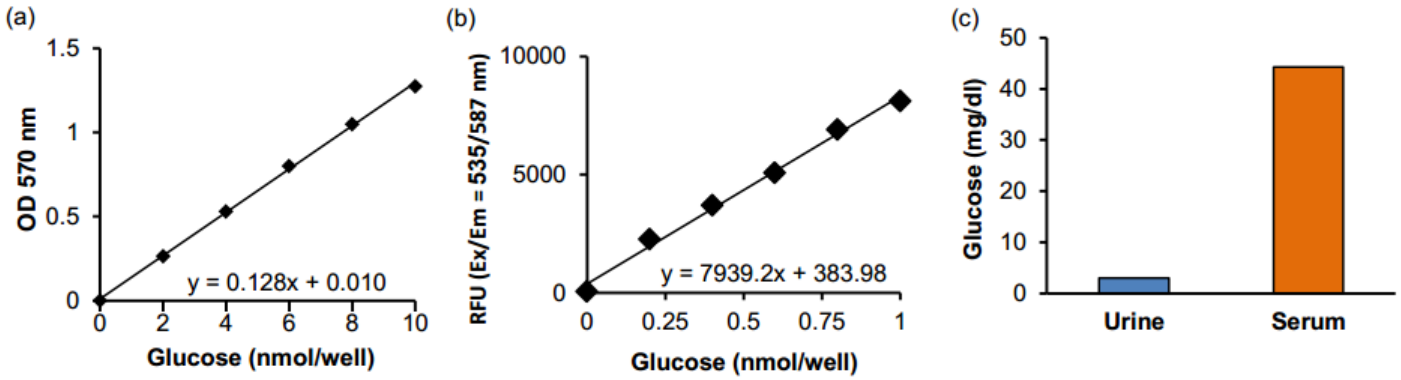

Figure: Glucose Standard Curve; (a) Colorimetric (b) Fluorometric, (c) Quantitation of Glucose in human urine & serum. Urine & serum samples were deproteinized using a 10 kDa Spin Column (10000xg, 10 min, 4°C). Urine filtrate (20 µl) & serum filtrate (1 µl) were spiked with a known amount of glucose as internal standard (4 nmol). Assays were performed according to the kit protocol. Calculated concentrations: Urine: 3.00 ± 0.4 mg/dl; Serum: 44.2 ± 6.7 mg/dl.

Frequently Asked Questions

1. Will the phenol red in the media affect the assay readout?

Very low amounts of media are used for each sample. This will generate a very low background at the best. Please use only media as a background control and subtract this reading from all sample readings to accommodate for the phenol red.

2. Why does the final color development starts as pink, then goes to brown then disappears?

This is a very common phenomenon observed with use of the oxired probe and is caused due to excessive analyte concentration in the samples. Therefore, if your glucose samples are too concentrated, you may see this. Please dilute your samples with the assay buffer before reanalyzing.

3. 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.

4. 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 kit's citations to see what kind of samples have been used with this kit other than mammalian samples.

5. 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.

6. 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.

7. 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.

8. 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.

9. 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.

10. 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.

11. Does high sucrose concentration in the sample affect this assay's performance?

Yes, we have data comparing glucose in presence and absence of high sucrose (0.25 M and 1 M-effect almost identical) showing that the sucrose conc affects the assay. Therefore, this kit is not recommended to be used with samples.

12. 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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