Glucose Assay Kit III

Ultra-sensitive, high-throughput adaptable fluorometric assay to determine glucose in various biological samples

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K688
Description	Glucose is the main energy source for virtually all living organisms. Glucose level is a key diagnostic parameter for many metabolic disorders. Measurement of glucose can be very important in both research and drug discovery processes. PromoKine's Glucose Assay Kit III is simple, rapid, ultra-sensitive and suitable for high-throughput. In this assay, D-glucose is enzymatically oxidized to form a product which reacts with a colorless probe to generate a fluorescent signal (Ex/Em = 535/587 nm). The fluorescence generated is directly proportional to the amount of glucose. This assay kit can detect less than 0.5 µM glucose in various biological samples. D-Glucose —*Glucose Enzyme Mix*→ Product —*FluoProbe + Glucose Substrate Mix*→ Fluorescence detection (Ex/Em = 535/587 nm)
Quantity	100 assays
Applications	• Measurement of glucose in various tissues/cells • Analysis of metabolism and cell signaling • Mechanistic study of obesity and diabetes
Sample Type	• Serum, plasma & other body fluids • Animal tissues: Liver, muscle, heart etc. • Cell culture: Adherent or suspension cells • Growth media • Food

Components	**Quantity**	**Color Code**
Glucose Assay Buffer	25 ml	WM
FluoProbe (in DMSO)	0.4 ml	Blue
Glucose Substrate Mix (lyophilized)	1 vial	Red
Glucose Standard (100 mM)	100 µl	Yellow
Glucose Enzyme Mix (lyophilized)	1 vial	Green

User Supplied Reagents & Equipment	• 96-well plate (white or black) with flat bottom • Multi-well spectrofluorometer (fluorescence microplate reader)
Storage and Reagents Preparation	Store kit at –20°C, protected from light. Warm the Glucose Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. • **FluoProbe**: 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.
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 minutes. 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. Note: 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 spin column (Cat.No. PK-CA577-1997). 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
FluoProbe	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/=l = 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

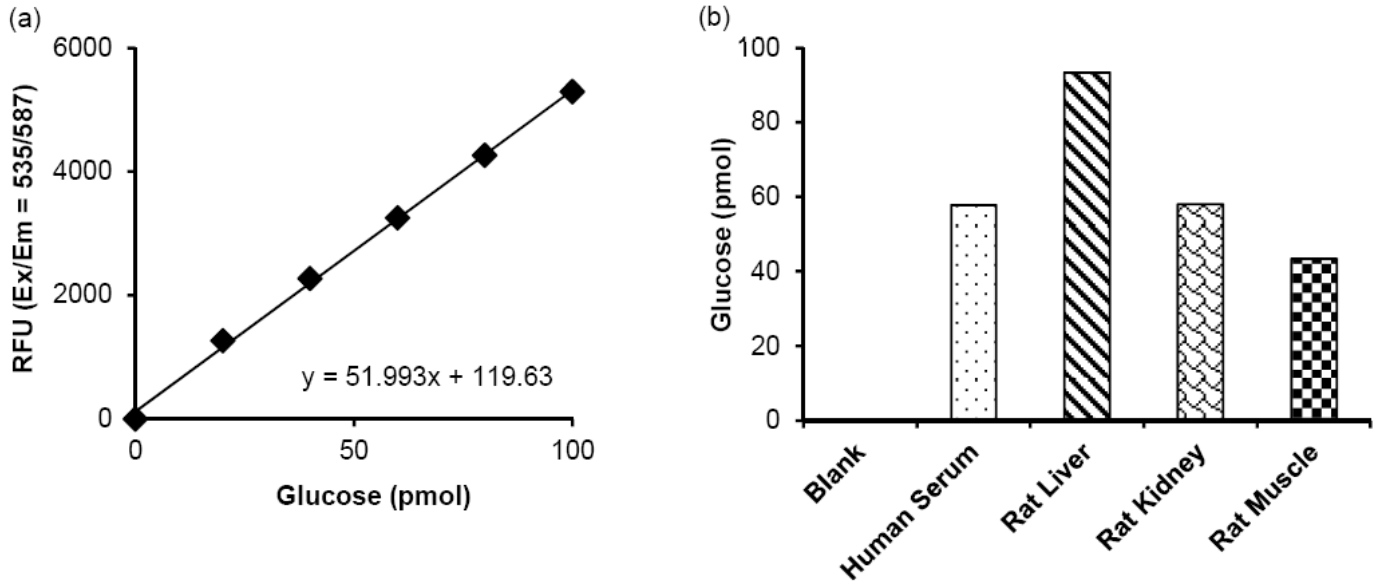

Figure: (a) Glucose Standard curve (b) Measurement of Glucose levels in human serum (1 µl of 1:10 diluted) & rat tissue lysates from liver, kidney & muscle (0.14 µg, 0.19 µg & 0.93 µg respectively).

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