

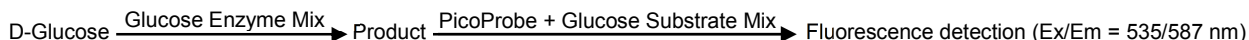
# PicoProbe™ Glucose Fluorometric Assay Kit

rev. 8/13

(Catalog # K688-100; 100 assays; Store at -20°C)

## I. Introduction:

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. Biovision's PicoProbe™ Glucose Assay kit 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 the fluorescence (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.



## II. Application:

- Measurement of glucose in various tissues/cells
- Analysis of metabolism and cell signaling
- Mechanistic study of obesity and diabetes

## III. Sample Type:

- Serum, plasma & other body fluids
- Animal tissues: liver, muscle, heart etc.
- Cell culture: adherent or suspension cells
- Growth media
- Food

## IV. Kit Contents:

Components	K688-100	Cap Code	Part Number
Glucose Assay Buffer	25 ml	WM	K688-100-1
PicoProbe™ (in DMSO)	0.4 ml	Blue	K688-100-2
Glucose Enzyme Mix (Lyophilized)	1 vial	Green	K688-100-3
Glucose Substrate Mix (Lyophilized)	1 vial	Red	K688-100-4
Glucose Standard (100 mM)	100 μl	Yellow	K688-100-5

## 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<sub>2</sub>O. 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 ( $1 \times 10^6$ ) 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 (BioVision, Cat # K808-200) or by spin filtering through a 10kD membrane (BioVision # 1997-25).
  - 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<sub>2</sub>O, 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<sub>2</sub>O. 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 $\mu$ l	47 $\mu$ l
PicoProbe™	1 $\mu$ l	1 $\mu$ l
Glucose Enzyme Mix	2 $\mu$ l	---
Glucose Substrate Mix	2 $\mu$ l	2 $\mu$ l

Add 50  $\mu$ l of the Reaction Mix to each well containing the Standard & test samples. Mix well.

**Note:** For samples having high NADH levels, add 50  $\mu$ l 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.

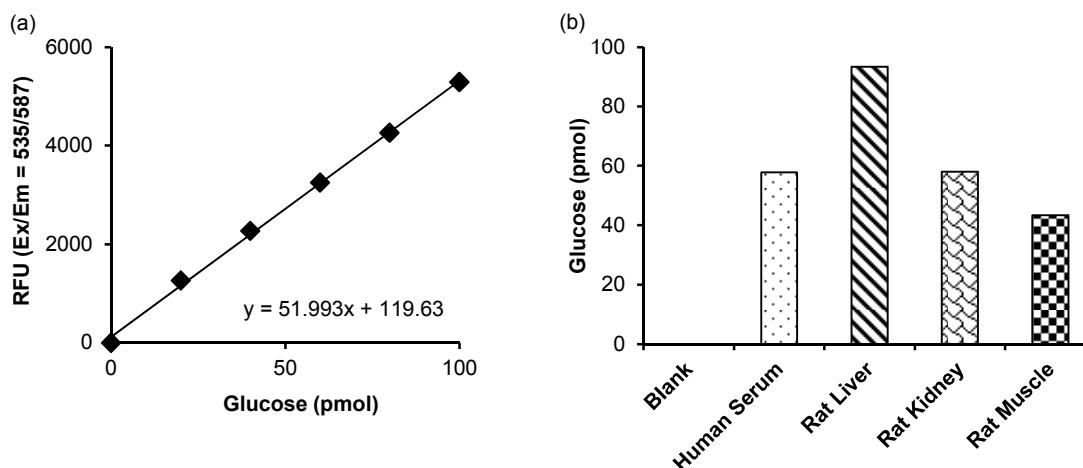
$$\text{Sample Glucose concentration} = B/V \times \text{Dilution Factor} = \text{pmol}/\mu\text{l} = \text{nmol}/\text{ml} \text{ or } \mu\text{M}$$

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

**V** = sample volume added in the reaction well ( $\mu$ l)

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  $\mu$ l of 1:10 diluted) & rat tissue lysates from liver, kidney & muscle (0.14  $\mu$ g, 0.19  $\mu$ g & 0.93  $\mu$ g respectively).

#### IX. RELATED PRODUCTS:

Glucose Assay Kit  
 Glucose Assay Kit II  
 Glucose and Sucrose Assay Kit  
 Glucose Uptake Fluorometric Assay Kit  
 Glucose Uptake Colorimetric Assay kit  
 Glucose Dehydrogenase Activity Assay Kit  
 Glucose Oxidase Activity Assay Kit  
 Glucose-1-Phosphate Colorimetric Assay Kit  
 Glucose-6-Phosphate Assay Kit  
 Glucose-6-Phosphate Dehydrogenase Assay Kit

PicoProbe™ Glucose-6-Phosphate Assay Kit  
 Lactate Assay Kit  
 Phosphoglucomutase Colorimetric Assay Kit  
 Phosphoglucose Isomerase Colorimetric Assay Kit  
 Maltose and Glucose Assay Kit  
 NAD/NADH Quantification Kit  
 NADP/NADPH Quantification Kit  
 Glycogen Assay Kit  
 Hexokinase Colorimetric Assay Kit  
 Starch Assay Kit

**FOR RESEARCH USE ONLY! Not to be used on humans.**