Glucose

Fluorometric Microplate Assay Kit

User Manual

Catalog # CAK8003

(Version 1.1A)

Detection and Quantification of Glucose Content in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Glucose ($C_6H_{12}O_6$) is a key diagnostic parameter for many metabolic disorders. Increased glucose levels have been associated with diabetes mellitus, hyperactivity of thyroid, pituitary and adrenal glands. Decreased levels are found in insulin secreting tumors, myxedema, hypopituitarism and hypoadrenalism.

Glucose Fluorometric Microplate Assay Kit provides a simple and sensitive method for monitoring glucose content in various samples. In this assay, glucose oxidase specifically oxidizes free glucose generating a compound that reacts with the glucose probe, which can be detected fluorometrically (Ex/Em 535/587).

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Black Microplate	1 plate	
Reaction Buffer	20 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Probe	Powder x 1	-20 °C, keep in dark
Probe Diluent	1 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Enzyme: add 1 ml Reaction Buffer to dissolve before use. Aliquot & store at -20 °C. Use within one month.

Probe: Warm Probe Diluent to RT prior to use to melt frozen Probe Diluent; then add 1 ml Probe Diluent to dissolve. Store at -20 °C, protect from light and moisture. Use within one month.

Standard: add 1 ml distilled water to dissolve before use; then add 2 µl into 998 µl distilled water, the concentration will be 100 µmol/L. Store at -20 °C. Use within one month.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Fluorescence microplate reader to read fluorescence at Ex/Em = 535/587
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml distilled water, put it in the boiling water bath for 15 minutes, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For liquid samples

Detect directly or dilute with distilled water.

V. ASSAY PROCEDURE

Warm the solution to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	170 µl	170 µl	170 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Probe	10 µl	10 µl	10 µl
Enzyme	10 µl	10 µl	10 µl
Mix, put it in the oven, 37 °C for 15 minutes, protected from light, record fluorescence measured at Ex/Em = 535/587 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the weight of sample

$$\text{Glucose (μmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Water})$$

$$= 0.1 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

2. According to the volume of sample

$$\text{Glucose (μmol/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 0.1 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

W: the weight of sample, g;

$C_{Standard}$: the Standard concentration, 100 μmol/L = 0.1 μmol/ml;

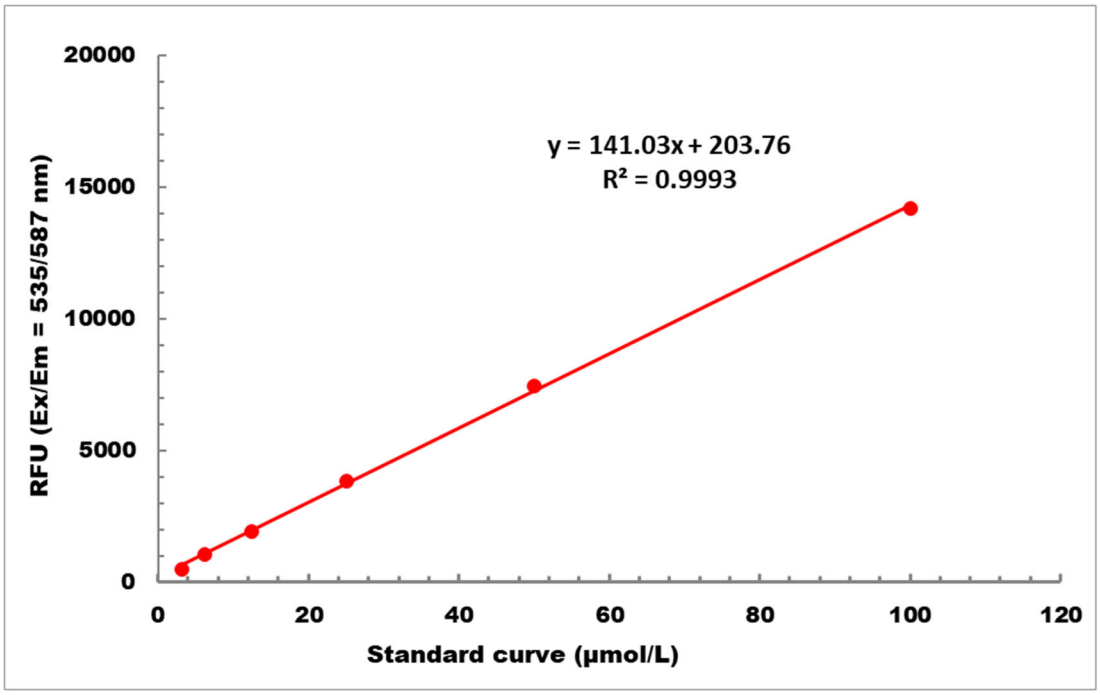
$V_{Standard}$: the volume of sample, 0.01 ml;

V_{Sample}: the volume of sample, 0.01 ml;

V_{Water}: the volume of distilled water, 1 ml.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 5 µmol/L - 100 µmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES