Glucose

Fluorometric Microplate Assay Kit

User Manual

Catalog # CAK8003

(Version 1.2A)

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 glucosecontent 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 mlx 1	4 °C
Enzyme	Powder x 1	-20 °C, keep in dark
Probe	Powder x 1	-20 °C, keep in dark
Probe Diluent	1 mlx 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 mldistilled waterto generate 50 mmol/L of standardstock solution,store at 4 °C for 1 monthor -20°C for 1 year after reconstitution.Then dilute to100 µmol/Lstandard top solutionby adding 2 µl stock solution into 998µl distilled water.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be100/50/25/12.5/6.25/3.12µmol/L.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use.Aliquot & store at -20 °C.Use within 1 month.

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

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For tissue samples

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

2. For liquid samples

Detect directly or dilute with distilled water.

VI. ASSAY PROCEDURE

Warm thesolution 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:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(RFU_{Sample}-RFU_{Blank})-Intercept}{Slope}\times n(\mu mol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard RFU and concentration

2.1 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(RFU_{Sample}-RFU_{Blank})}{(RFU_{Standard}-RFU_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu mol/g)$$

2.2According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(RFU_{Sample}-RFU_{Blank})}{(RFU_{Standard}-RFU_{Blank})\times V_{Sample}}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, μmol/L

$V_{Standard}$: the volume of standard in assay procedure, μl

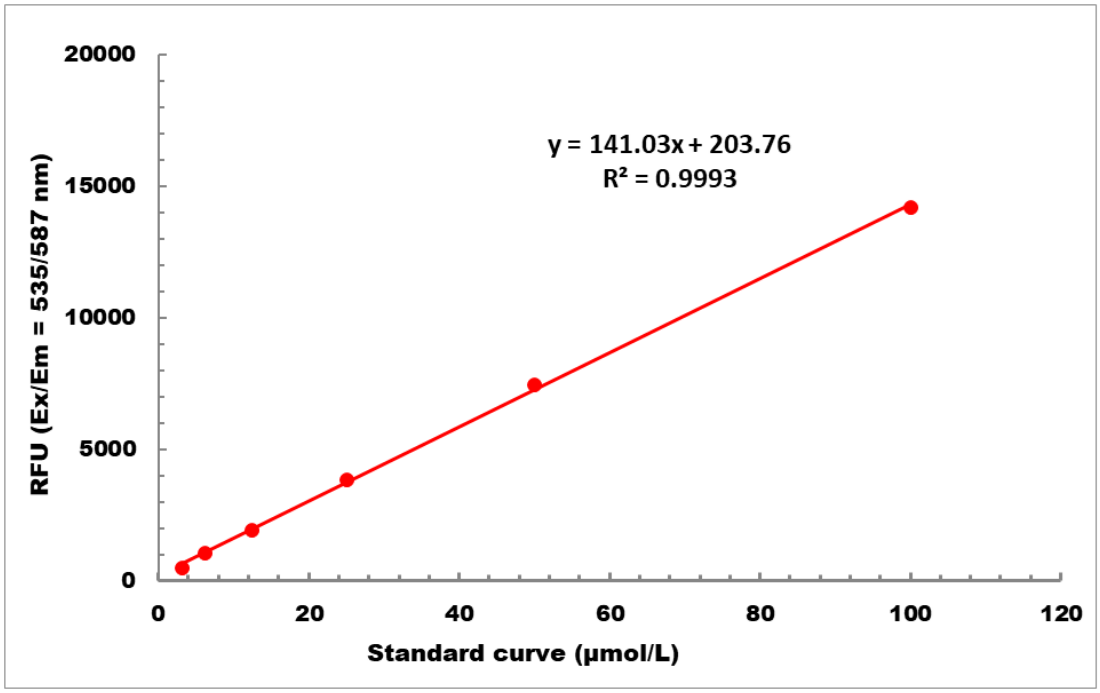
V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer, μl

W: the weight of sample, g

VIII. TYPICAL DATA

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



Detection Range: 5µmol/L - 100µmol/L