Glucose Microplate Assay Kit

User Manual

Catalog # CAK1025

(Version 1.2C)

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.

The assay is initiated with the enzymatic catalysis of glucose by glucose oxidase. The enzyme catalysed reaction products H_2O_2 react with the substrate, and can be measured at a colorimetric readout at 505 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Enzyme	Powder x 1	-20 °C
Enzyme Diluent	10 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

Enzyme: add 10 ml Enzyme Diluent to dissolve before use.

Dye Reagent: add 10 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 10 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 505 nm
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.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Enzyme	90 µl	90 µl	90 µl
Dye Reagent	90 µl	90 µl	90 µl
Mix, put it in the oven, 37 °C for 15 minutes, record absorbance measured at 505 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

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

$$= 10 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

2. According to the volume of sample

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

$$= 10 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

W: the weight of sample, g;

$C_{Standard}$: the Standard concentration, 10 mmol/L = 10 μmol/ml;

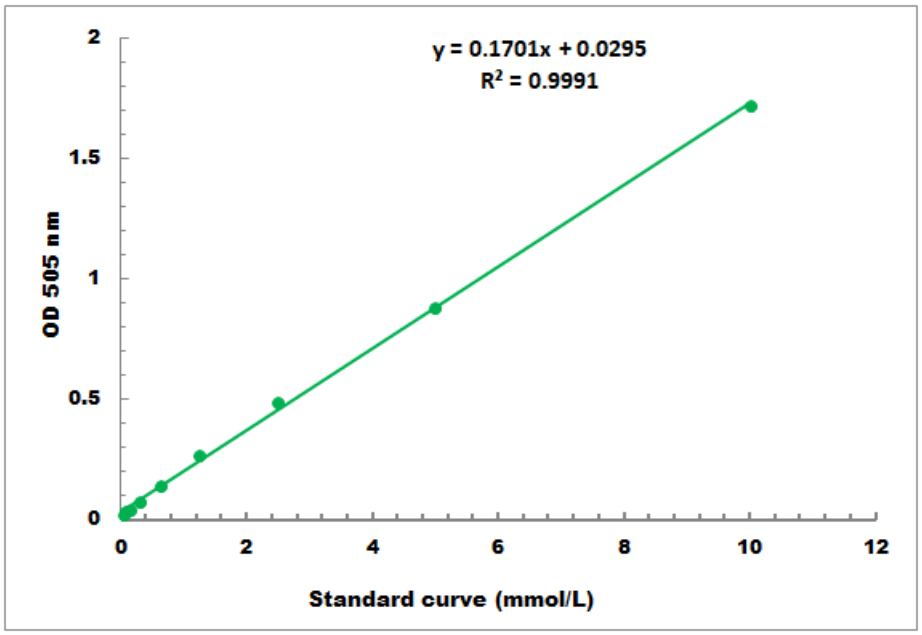
$V_{Standard}$: the volume of sample, 0.02 ml;

V_{Sample}: the volume of sample, 0.02 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: 0.1 mmol/L - 10 mmol/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