Glucose (Tissue) Microplate Assay Kit

Cat #: orb390731 (manual)

For Research Use Only. Not For Use in Diagnostic Procedures!

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

PRINCIPLE OF THE ASSAY

Glucose (C6H12O6) 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 tumours, myxedema, hypopituitarism and hypoadrenalism.

Glucose (Tissue) Microplate Assay Kit provides a simple and sensitive method for monitoring glucose concentration in various samples. The assay is initiated with the enzymatic catalysis of glucose by glucose oxidase. The enzyme catalysed reaction product reacts with the dye and can be measured at a colorimetric readout at 505 nm.

MATERIALS PROVIDED

Component	Volume	Storage
96-Well Microplate	1 plate	
Enzyme	Powderx 1	-20 °C
Enzyme Diluent	10 mlx 1	4 °C
Dye Reagent	Powderx 1	4 °C, keep in dark
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 Strips	
Manual	1 Manual	

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OTHER SUPPLIES REQUIRED

- Microplate reader to read absorbance at 505 nm
- Distilled water
- Pipettor, multi-channel pipettor
- Pipette tips
- Mortar
- Ice
- Centrifuge
- Timer

REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in1 ml distilled water to generate 50 mmol/L of standard stock solution, store at 4 °C for 1 month after reconstitution. Then dilute to10mmol/L standard top solution by adding0.2 ml stock solution into 0.8 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be10/5/2.5/1.25/0.625/0.312/0.156 mmol/L.

Enzyme: Add 10 ml Enzyme Diluent to dissolve before use. Store at 4 °C for 1 day or

-20°C for 1 month after reconstitution.

Dye Reagent: Add 10 ml distilled water to dissolve before use. Keep in dark and store at 4 °C for 1 week or -20°C for 1 month after reconstitution.

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

SAMPLE COLLECTION AND STORAGE

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 10minutes, take the supernatant into a new centrifuge tube for detection.

2. For liquid samples

Detect directly

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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:

*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.

CALCULATION OF RESULTS

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

$$C=\frac{(OD_{Sample}-OD_{Blank})-Intercept}{Slope}\times\frac{V_{Standard}}{V_{Sample}}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

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

2.1 According to the protein concentration of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times C_{Protein}\times V_{Sample}}(\mu mol/mg)$$

2.2 According to the weight of sample

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

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2.3 According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

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

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

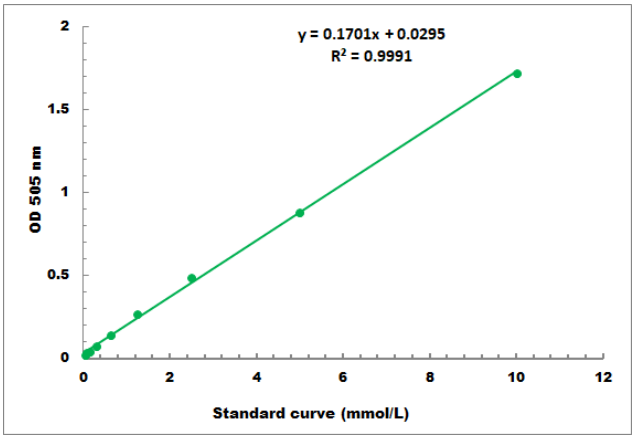
V_{Sample}: the volume of sample in assay procedure, μl

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

$C_{Protein}$: the sample protein concentration, mg/ml W: the weight of sample, g

TYPICAL DATA

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



Detection Range: 0.1 mmol/L - 10mmol/L

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