Tissue Glucose Assay Kit

Tissue Glucose Assay Kit is a detection kit for the quantification of Glucose Content in tissue extracts and cell lysate.

Catalog number: ARG82020

Package: 96 wells

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INTRODUCTION

Glucose is a simple sugar with the molecular formula $C_6H_{12}O_6$. Glucose is the most abundant monosaccharide, a subcategory of carbohydrates. Glucose is mainly made by plants and most algae during photosynthesis from water and carbon dioxide, using energy from sunlight, where it is used to make cellulose in cell walls, the most abundant carbohydrate in the world. In energy metabolism, glucose is the most important source of energy in all organisms. Glucose for metabolism is stored as a polymer, in plants mainly as starch and amylopectin, and in animals as glycogen. Glucose circulates in the blood of animals as blood sugar. The naturally occurring form of glucose is d-glucose, while l-glucose is produced synthetically in comparatively small amounts and is of lesser importance. Glucose is a monosaccharide containing six carbon atoms and an aldehyde group, and is therefore an aldohexose. The glucose molecule can exist in an open-chain (acyclic) as well as ring (cyclic) form. Glucose is naturally occurring and is found in fruits and other parts of plants in its free state. In animals, glucose is released from the breakdown of glycogen in a process known as glycogenolysis.

Glucose, as intravenous sugar solution, is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system. It is also on the list in combination with sodium chloride. [Provide by Wikipedia: Glucose]

PRINCIPLE OF THE ASSAY

This Tissue Glucose Assay Kit is a simple colorimetric assay that measures the amount of Glucose present in tissue extracts and cell lysate. The assay is based on the enzyme driven reaction. 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. The concentration of Glucose in the samples is then determined by comparing the O.D. 505 nm absorbance of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
96 Well microplate	1 plate	RT
Enzyme (lyophilized)	1 vial	-20°C
Enzyme Diluent	10 mL (ready to use)	4°C
Dye Reagent (lyophilized)	1 vial	4°C (protect from light)
Standards (lyophilized)	1 vial	4°C
Plate sealer	3 ea	RT
Technical Manual	1 ea	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 505 nm
- Centrifuge
- Mortar
- Deionized or Distilled water
- Ice
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue samples: Weigh out 0.1 g tissue, homogenize with 1 mL of distilled water, and put it in the boiling water bath for 15 minutes. Centrifuged at 8,000 x g for 10 minutes at 4°C. Take the supernatant into a new centrifuge tube for detection.

REAGENT PREPARATION

- Enzyme: Add 10 mL of Enzyme Diluent to dissolve before use.
- Dye Reagent: Add 10 mL of distilled water to dissolve before use.
- Standards: Add 1 mL of distilled water to dissolve before use, mix well, and add 0.2 mL into 0.8 mL of distilled water. The concentration will be 10 mmol/L. Use the 10 mmol/L Standards to prepare a series of diluted standards according to the Table below.

Standard tube	Final Standard conc. (mmol/L)	distilled water (μL)	Volume of 10 mmol/L Standards (μL)
S1	10	0	500
S2	5	250	250 of S1
S3	2.5	250	250 of S2
S4	1.25	250	250 of S3
S5	0.625	250	250 of S4
S6	0.313	250	250 of S5
S7	0.156	250	250 of S6
S0	0	250	0

ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

Add following reagents into the 96 Well Microplate:			
Reagent	Sample	Standards	Blank
Sample	20 µL		
Standards		20 µL	
Distilled water			20 µL
Enzyme	90 µL	90 µL	90 µL
Dye Reagent	90 µL	90 µL	90 µL
Mix well, and incubate for 15 minutes at 37°C in the oven. Read the absorbance at O.D. 505 nm.			

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Control, Blank and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. According to the weight of sample:

Glucose (μmol/g)

$= [(C_{Standaard} \times V_{Standardl}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / (V_{Sample} \times W / V_{Water})$

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

5. According to the volume of sample:

Glucose (μmol/mL)

$= [(C_{Standaard} \times V_{Standardl}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / V_{Sample}$

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

Note:

W: the weight of sample, g;

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

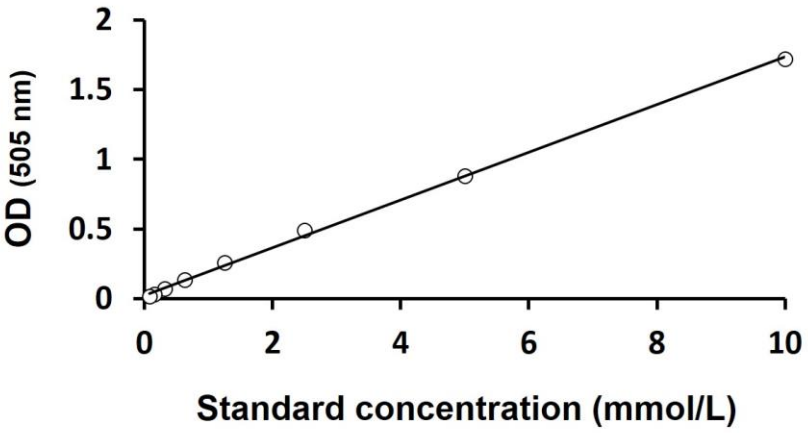
V_{water}: the total volume of distilled water, 1 mL;

V_{Sample}: the volume of sample, 0.02 mL;

$V_{standard}$: the volume of Standard, 0.02 mL.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Tissue Glucose Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.1 mmol/L