USER GUIDE

For Research Use Only. Not For Use In Diagnostic Procedures

Version 2.0

葡萄糖检测试剂盒

Glucose Assay Kit

Do not eat Store at -20° C & in the dark.

Cat.No.MKG3845 **Size :** 100 tests

Technical literature is available at : www.mesgenbio.com
E-mail MesGen Technical Services if you have questions on use of this system : tech@mesgenbio.com

Application

For quantitative determination of glucose and evaluation of drug effects on glucose metabolism.

Description

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. Simple, direct and high-throughput assays for measuring glucose concentrations find wide applications in research and drug discovery. MesGen Biotechnology glucose assay kit uses a single Working Reagent that combines the glucose oxidase reaction and color reaction in one step. The color intensity of the reaction product at 570nm or fluorescence intensity at λex/em = 530/585nm is directly proportional to glucose concentration in the sample.

Key Features

Sensitive and accurate. Use as little as 10 µL samples. Linear detection range in 96-well plate: 10 to 1250 µM glucose for colorimetric assays and 1 to 50 µM for fluorimetric assays.
Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 5-10 min at room temperature.

Samples

Serum, plasma, urine, saliva, milk, culture medium, food, agriculture etc

Kit contents

Reagent A : 5 mL **Reagent B : 5** mL **Reagent C : 200** µL
10mM **Glucose Standard:** 2 mL

Colorimetric procedure

Note:
(1) *glycerol and SH-containing reagents (e.g. β–mercaptoethanol, dithiothreitol) are known to interfere in this assay and should be avoided in sample preparation.*
(2) *This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to standard and samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.*

1. Equilibrate all components to room temperature. During experiment, keep reconstituted Reagent in a refrigerator or on ice.
2. *Standards and samples*: prepare 400 µL 2000 µM Standard by mixing 200 µL 10 mM standard with 800 µL dH_2O. Dilute standard in dH_2O as follows.

No.	2000 µM STD + H_2O	Vol (µL)	Glucose (µM)
1	100 + 0	100	2000
2	80 + 20	100	1600
3	60 + 40	100	1200
4	40 +60	100	800
5	10 + 90	100	200
6	5 + 95	100	100
7	2 + 98	100	40
8	0.5 + 95.5	100	10
9	0 + 100	100	0

Transfer 10 µL standards and 10 µL samples into separate wells of a clear flat-bottom 96-well plate.

3. *Reaction*. For each reaction well, mix 50 µL Reagent A, 50 µL Reagent B and and 2 µL Reagent C (*vortex briefly before pipetting*). Transfer 100 µL Working Reagent into each reaction well. Tap plate to mix.Incubate 10-15 min at room temperature.
4. Read optical density at 570nm (550-585nm).

Fluorimetric procedure

For fluorimetric assays, the linear detection range is 10 to 100 µM galactose. Prepare 100 µM galactose standard by mixing 10 µL 10 mM standard with 990 µL H_2O.
Then dilute standards in H_2O (see *Colorimetric Procedure*) to 100, 80, 60, 40, 30, 20, 10 and 0 µM.

1. Transfer 10 µL standards and 10 µL samples into separate wells of a *black* 96-well plate.
2. Add 100 µL Working Reagent, tap plate to mix. Incubate 10-15

min.

3. Read fluorescence at λex = 530nm and λem = 585nm.

Notes: If the calculated galactose concentration of a sample is higher than 1000 μM in colorimetric assay or 100 μM in fluorimetric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor n.

Calculation

Subtract blank value from the standard values and plot the ΔOD or ΔRFU against standard concentrations. Determine the slope and calculate the galactose concentration of Sample,

$$\textit{Colorimetry:}\quad [\text{Galactose}] = \frac{OD_{SAMPLE} - OD_{H2O}}{\text{Slope}} \times n \quad (\mu M)$$

$$\textit{Fluorimetry:}\quad [\text{Galactose}] = \frac{RFU_{SAMPLE} - RFU_{H2O}}{\text{Slope}} \times n \quad (\mu M)$$

OD_{SAMPLE}, OD_{H2O} are optical density values of the sample and water.RFU_{SAMPLE}, RFU_{H2O} are fluorescence intensity values of the sample and water. n is the dilution factor.

Conversions: 1 mM galactose equals 18 mg/dL, 0.018% or 180 ppm.

Storage conditions

The kit is shipped on dry ice. Store all components at -20°C.

Shelf life

6 months after receipt.

Precautions

Reagents are for research use only.

Normal precautions for laboratory reagents should be exercised while using the reagents.

Please refer to Material Safety Data Sheet for detailed information.

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