Technical Bulletin

High Sensitivity Glucose Quantitation Kit

Catalogue number MAK543

Product Description

Glucose, a monosaccharide, is the most important carbohydrate in biology. It is a source of energy and metabolic intermediate for cell growth. Glucose is one of the main products of photosynthesis and starts cellular respiration in both prokaryotes and eukaryotes. The Glucose Quantitation Assay Kit provides a quick and sensitive method for the measurement of glucose in various biological samples (such as serum, plasma, body fluid, food, growth medium, etc.)

Components

The kit is sufficient for 500 colorimetric assays in 96-well plates.

- Peroxidase Substrate — 1 Vial
  Catalogue Number MAK543A
- Assay Buffer — 50 mL
  Catalogue Number MAK543B
- Horseradish Peroxidase (HRP) — 1 Vial
  Catalogue Number MAK543C
- Glucose Oxidase — 1 Vial
  Catalogue Number MAK543D
- DMSO — 200 µL
  Catalogue Number MAK543E
- Glucose Standard — 1 Vial
  Catalogue Number MAK543F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Fluorescence multiwell plate reader.
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

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

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Equilibrate reagents to room temperature prior use.

Procedure

All Samples and Standards should be run in duplicate.

Preparation of Stock Solutions

Peroxidase Substrate Stock Solution (250X): Add 100 µL of DMSO into the vial of Peroxidase Substrate.

Note: The Peroxidase Substrate is unstable in the presence of thiols such as dithiothreitol (DTT) and 2-mercaptoethanol. The final concentration of DTT or 2-mercaptoethanol in the reaction should be no higher than 10 µM. The Peroxidase Substrate is also unstable at high pH (> 8.5). Therefore, the reaction should be performed at pH 7–8. The provided assay buffer (pH 7.4) is recommended.

Horseradish Peroxidase (HRP) Stock Solution (10 U/mL): Add 1 mL of Assay Buffer into the vial of Horseradish Peroxidase.

Glucose Oxidase Solution (100 U/mL): Add 1 mL of Assay Buffer into the vial of Glucose Oxidase.

Glucose Standard Stock Solution (800 mM): Add 1 mL of Assay Buffer into the vial of Glucose Standard.

Note: Avoid repeated freeze-thaw cycles. Store all the unused stock solutions in single use aliquots at -20 °C.

Preparation of Glucose Standards

1. Dilute the appropriate amount of the 800 mM Glucose Standard stock solution into Assay Buffer to produce a starting concentration of 30 µM.
2. Perform 1:3 serial dilutions in Assay Buffer to get the 10, 3, 1, 0.3, 0.1 and 0.03 µM serially diluted glucose standards (GS) shown in Table 1. Use Assay Buffer as the blank control.

Table 1

Serial dilution of Glucose Standards

Dilution	Glucose Std Vol (µL)	Assay Buffer Vol (µL)	Serial Dilution Source	Conc. (µM)
GS1	300	-	30 µM stock	30
GS2	75	150	From GS1	10
GS3	75	150	From GS2	3.33
GS4	75	150	From GS3	1.11
GS5	75	150	From GS4	0.37
GS6	75	150	From GS5	0.12
GS7	75	150	From GS6	0.04

Preparation of Assay Working Solution

Prepare Assay Working Solution as shown in Table 2 to a final volume of 5 mL. This is sufficient for one 96-well plate.

Table 2

Assay Working Solution Preparation

Components	Volume
Peroxidase Substrate	20 µL
HRP Stock Solution (10 U/mL)	100 µL
Glucose Oxidase Solution (100 U/mL)	100 µL
Assay Buffer	4.78 mL

Assay Reaction

1. Add 50 µL of each glucose standard, blank, and test sample into a black, flat bottom 96-well plate.
2. Add 50 µL of Assay Working Solution into each well of standard, blank, and test sample to make the total assay volume of 100 µL/well.
3. Incubate the reaction for 10 to 30 minutes at 37 °C, protected from light.

Note: High concentrations of glucose (such as, 100 µM in test sample or standard) may cause reduced fluorescence signal due to the overoxidation of the substrate.

Measurement

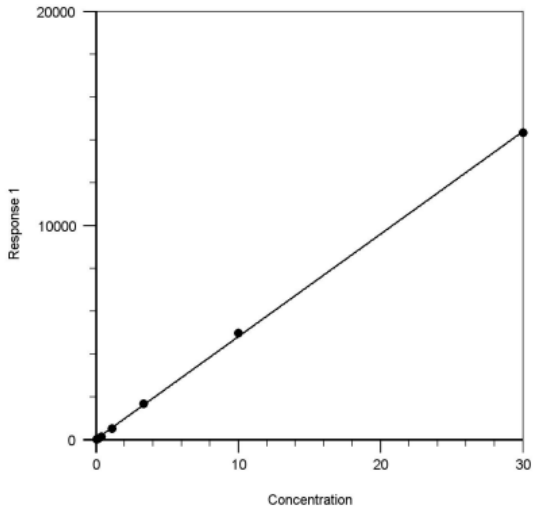
Monitor the fluorescence intensity with a fluorescence plate reader at $\lambda_{Ex/Em}$= 530-570 nm/590-600 nm (optimal $\lambda_{Ex/Em}$ = 540/590 nm).

Results

1. The reading (RFU) obtained from the blank well is used as a negative control.
2. Subtract the blank value from the standards to obtain the baseline corrected values.
3. Plot the standards readings to obtain a standard curve and equation.
4. The concentration of glucose in the test samples may be determined from the standard curve.

Figure 1

Typical Glucose Standard Curve



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