Technical Bulletin

Glucose Assay Kit

Catalogue number MAK476

Product 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 and 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. The 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 570 nm or fluorescence intensity at λ_{Em} = 585 nm/λ_{Ex} = 530 nm is directly proportional to glucose concentration in the sample.

The linear detection range of glucose for the colorimetric assay is 5 – 300 µM (90 µg/dL - 5.4 mg/dL) and 1 – 30 µM for the fluorometric assay.

The kit is suitable for glucose determination in serum, plasma, milk, culture medium, food, beverages, and other biological preparations. Note: MAK476 is not compatible with urine samples. Please use an alternate kit for urine glucose determination.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer — 10 mL
  Catalogue Number MAK476A
- Enzyme Mix — 120 µL
  Catalogue Number MAK476B
- Dye Reagent — 120 µL
  Catalogue Number MAK476C
- Standard (300 mg/dL Glucose) — 1 mL
  Catalogue Number MAK476D

Equipment Required but Not Provided

- Pipetting devices and accessories (such as, multichannel pipettor, pipette tips, etc.)
- Multiwell plate reader.
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- Microcentrifuge capable of RCF ≥ 14,000 × g.

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

Equilibrate all components to room temperature. Keep thawed Enzyme on ice during assay.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

Note: Samples can be analyzed immediately after collection or stored in aliquots at -20 °C. Avoid repeated freeze-thaw cycles. If particulates are present, centrifuge sample and use clear supernatant for assay.

Saliva

Saliva samples should be centrifuged for 5 minutes at 14,000 × g prior to assay.

Milk

1. Milk samples should be cleared by mixing 100 µL of 6N HCl and 600 µL of milk.
2. Centrifuge for 5 minutes at 14,000× g and transfer the supernatant into a clean tube.
3. Add 170 µL of 6N NaOH per mL of supernatant.
4. Mix well and centrifuge at 14,000 × g for 5 minutes. Retain the supernatant for assay.
5. The dilution factor in this procedure is n = 1.36.

Phenol Red Culture Medium

1. To determine glucose in phenol red culture medium, dilute the sample in the same glucose free medium for colorimetric assay.
2. For fluorometric assay, dilute sample 20-fold or more in purified water.

All Samples

Transfer 20 µL of prepared Sample to wells of a 96-well plate.

Colorimetric Standard Curve Preparation

1. Prepare a 300 µM Glucose Standard by mixing 15 µL of the 300 mg/dL Glucose Standard with 818 µL of purified water.
   a. If determining glucose in phenol red culture medium, prepare Standards in the phenol red medium rather than purified water.
2. Prepare Colorimetric Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Colorimetric Glucose Standards

Well	300 µM Standard	Purified Water	Glucose (µM)
1	200 µL	0 µL	300
2	120 µL	80 µL	180
3	60 µL	140 µL	90
4	-	200 µL	0

3. Mix well and transfer 20 µL of each Standard into separate wells of a clear 96-well plate.

Fluorometric Standard Curve Preparation

1. Prepare Glucose standards according to Colorimetric Standard Curve Preparation section.
   a. If determining glucose in phenol red culture medium, prepare Standards in the phenol red medium rather than purified water.
2. For all Fluorometric samples, including those in phenol red culture medium, mix 20 µL of the Standards from Colorimetric Procedure with 180 µL of purified water according to Table 2.

Table 2.
Preparation of Fluorometric Glucose Standards

Well	Colorimetric Standard	Purified Water	Glucose (µM)
1	20 µL of 300 µM Std	180 µL	30
2	20 µL of 180 µM Std	180 µL	18
3	20 µL of 90 µM Std	180 µL	9
4	-	200 µL	0

3. Mix well and transfer 20 µL of each Standard into separate wells of a black 96-well plate.

Working Reagents

Note: Vortex the Enzyme Mix briefly before pipetting.

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 87 µL of Working Reagent according to Table 3.

Table 3.
Preparation of Working Reagent

Reagent	Volume
Assay Buffer	85 µL
Enzyme Mix	1 µL
Dye Reagent	1 µL

2. Transfer 80 µL of Working Reagent into each Sample and Standard well. Tap plate to mix.

Measurement

1. Incubate at room temperature (protect plate from light for fluorometric assay) for 30 minutes.
2. Measure the absorbance (OD) at 570 nm or fluorescence (F) at λ_{Ex} = 530 nm and λ_{Em} = 585 nm.

Results

1. Calculate ΔOD or ΔF by subtracting the blank reading (OD or fluorescence intensity F) of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD or ΔF against standard concentrations and determine the slope of the standard curve.
3. Calculate the glucose concentration of Sample.

Glucose (µM) =

$$\frac{R_{SAMPLE}-R_{BLANK}}{\text{Slope } (\mu M^{-1})} \times DF$$

where:

R_{Sample} = OD or fluorescence intensity (F) reading of Sample

R_{Blank} = OD or fluorescence intensity (F) reading of Blank

DF = Sample dilution factor (DF = 1 for undiluted Samples)

If the calculated sample glucose concentration is higher than 300 µM in the colorimetric assay or 30 µM in the fluorometric assay, dilute Sample in purified water and repeat the assay. Multiply the result by the dilution factor.

Conversions: 1 mg/dL of glucose equals 55.5 µM, 0.001%, or 10 ppm.

Figure 1.
Typical Colorimetric Glucose Standard Curve

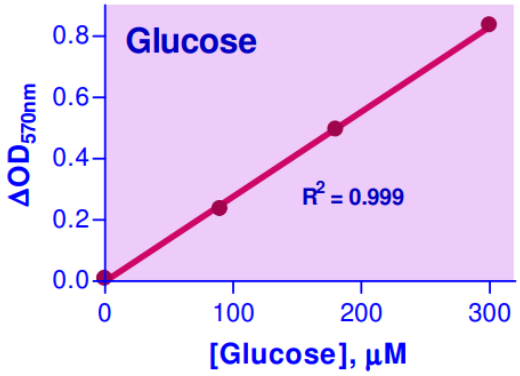
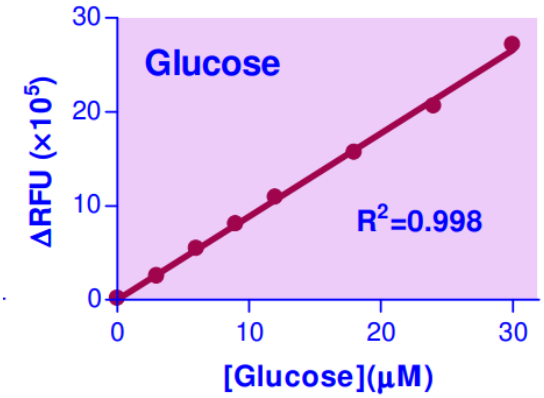


Figure 2.
Typical Fluorometric Glucose Standard Curve



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