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Product Information

Glucose and Sucrose Colorimetric/Fluorometric Assay Kit

Catalog Number **MAK013**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Sucrose is a disaccharide that is hydrolyzed to glucose and fructose by invertase. Glucose is the primary carbohydrate utilized for energy during cellular respiration. Upon cellular uptake, glucose can be shunted towards breakdown via glycolysis or polymerized for energy storage.

In this assay kit, glucose is oxidized via glucose oxidase resulting in a colorimetric (570 nm)/ fluorometric (λ_{ex} = 535 nm/λ_{em} = 587 nm) product, proportional to the glucose present. To measure sucrose, invertase is added to the reaction to convert the sucrose to glucose and fructose. The free glucose can be subtracted from the total glucose to give the concentration of sucrose present. This kit has a linear range of detection between 0.2–1.0 nmole of glucose for the fluorometric assay and 2–10 nmoles of glucose for the colorimetric assay.

This kit is suitable for use with various biological samples including serum, plasma, other body fluids, food, and growth media.

Components

The kit is sufficient for 100 assays in 96 well plates.

Glucose Assay Buffer Catalog Number MAK013A	25 mL
Glucose Probe, in DMSO Catalog Number MAK013B	0.2 mL
Invertase Catalog Number MAK013C	1 vl
Glucose Enzyme Mix Catalog Number MAK013D	1 vl
Sucrose Standard, 100 nmole/µL Catalog Number MAK013E	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader.

Precautions and Disclaimer

This product is 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.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Glucose Assay Buffer – Allow buffer to come to room temperature before use.

Glucose Probe – Ready-to-use as supplied. Allow the probe to come to room temperature before use. Store protected from light at –20 °C for use within 2 months. Upon thawing, the Glucose Probe is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Glucose Probe Solution 5 to 10-fold with Glucose Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Glucose Enzyme Mix and Invertase – Reconstitute each in 220 µL of Glucose Assay Buffer. Mix well by pipetting, then aliquot each and store protected from light at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

Storage/Stability

The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Sucrose Standards for Colorimetric Detection
Dilute 10 µL of the 100 nmole/µL Sucrose Standard Solution with 990 µL of Glucose Assay Buffer to prepare a 1 nmole/µL standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 nmole/µL Sucrose standard solution into a 96 well plate, generating 0 (assay blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Glucose Assay Buffer to each well to bring the volume to 50 µL.

Sucrose Standards for Fluorometric Detection
Prepare a 1 nmole/µL standard solution as for the Colorimetric Assay. Dilute 20 µL of the 1 nmole/µL standard solution with 180 µL of Glucose Assay Buffer to generate a 0.1 nmole/µL standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 nmole/µL Sucrose standard solution into a 96 well plate generating, 0 (assay blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add Glucose Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Liquid samples can be measured directly. Bring samples to a final volume of 50 µL with Glucose Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

For sucrose detection, set up duplicate wells for each sample in which glucose is measured. These wells will be used to determine total glucose.

Assay Reaction
1. Add 2 µL of Invertase to each of the sucrose samples and to the sucrose standards. Add 2 µL of Glucose Assay Buffer to the glucose sample. Incubate the plate at 37 °C for 30 minutes.

2. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	**Volume**
Glucose Assay Buffer	46 µL
Glucose Probe	2 µL
Glucose Enzyme Mix	2 µL

3. Add 50 µL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at 37 °C. Protect the plate from light during the incubation.

4. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 590 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (assay blank) Sucrose Standard. Correct for the background by subtracting the 0 (assay blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Sucrose standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Concentration of Glucose and Sucrose

$$S_a/S_v = C$$

S_a = Amount of Glucose in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of Glucose in sample

Glucose molecular weight: 180.16 g/mole

Sample Calculation

Amount of Glucose (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of Glucose in sample

$$5.84 \text{ nmole}/50 \text{ μL} = 0.1168 \text{ nmole/μL}$$

$$0.1168 \text{ nmole/μL} \times 180.16 \text{ ng/nmole} = 21.04 \text{ ng/μL}$$

Sucrose = Total Glucose – Free Glucose

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice Cold Assay Buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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