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Product Information

Sucrose Assay Kit

Catalog Number **MAK267**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Sucrose ($C_{12}H_{22}O_{11}$), also known as table sugar, is one of the most important fuel sources used to generate the universal energy molecule ATP. Sucrose is a disaccharide that is hydrolyzed to glucose and fructose by invertase.

The assay kit is suitable for sucrose detection in growth medium, food, serum, plasma, and other biological samples. Sucrose is converted to glucose and fructose by invertase. The glucose is then oxidized to generate a colorimetric (570 nm)/fluorometric (λ_{ex} = 535 nm/ λ_{em} = 587 nm) product. The kit can detect 0.0002–10 mM sucrose concentrations.

Components

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

Sucrose Assay Buffer Catalog Number MAK267A	25 mL
Sucrose Probe, in DMSO Catalog Number MAK2967B	0.2 mL
Invertase Catalog Number MAK267C	1 vl
Sucrose Enzyme Mix Catalog Number MAK2697D	1 vl
Sucrose Standard, (100 nmole/µL) Catalog Number MAK267E	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.

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

Sucrose Probe – Warm to room temperature to thaw the solution prior to use. Store protected from light and moisture at –20 °C. Use within 2 months. Upon thawing, the Sucrose Probe is ready-to-use as supplied.

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

Invertase - Reconstitute with 220 µL of Sucrose Assay Buffer. Mix well by pipetting (do not vortex), then aliquot and store at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Sucrose Enzyme Mix – Reconstitute with 220 µL of Sucrose Assay Buffer. Mix well by pipetting (do not vortex), then aliquot and store at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

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

Sucrose Standards for Fluorometric Detection
Prepare a 1 nmole/µL solution as for the colorimetric assay. Dilute 100 µL of the 1 nmole/µL solution with 900 µL of the Sucrose Assay Buffer to prepare a 0.1 nmole/µL solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 nmole/µL standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add Sucrose Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Both the colorimetric and fluorometric assays require 50 µL of sample for each reaction (well). Samples may be assayed directly.

Add 1–50 µL samples into wells of a 96 well plate. Bring samples to a final volume of 50 µL with Assay Buffer.

Note: For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the range of the standard curve.

Sucrose Conversion
Add 2 µL of Invertase to each sample and standard well to convert sucrose to glucose.

Glucose Background
Free glucose can interfere with the sucrose assay. If glucose is present in the sample, run a glucose background control without the Invertase. In the absence of Invertase, the assay detects free glucose only, not the sucrose. Subtract the free glucose background from the sample reading.

For samples containing glucose, prepare two wells/sample. To one well add 2 µL of Invertase to convert sucrose to glucose. To the other well, add 2 µL of Assay Buffer (without Invertase) as the glucose background.

Assay Reaction
1. 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	**Samples and Standards**
Sucrose Assay Buffer	46 µL
Sucrose Probe	2 µL
Sucrose Enzyme Mix	2 µL

2. Add 50 µL of the Master Reaction Mix to each sample and standard well. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate 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} = 587 nm).

Results

Calculations

The reagent background for either assay 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.

Correct for glucose background by subtracting the glucose background (without Invertase) from all sample readings (with Invertase). Apply sample value to the standard curve in order to calculate sucrose concentration.

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

Concentration of Sucrose

$$S_a/S_v = C$$

S_a = Amount of Sucrose in the unknown sample (nmole) from standard curve
S_v = Sample volume (μL) added into the wells
C = Concentration of Sucrose in sample

Sucrose molecular weight: 342.3 g/mole

Sample Calculation

Amount of Sucrose (S_a) = 5.84 nmole (from standard curve)
Sample volume (S_v) = 50.0 μL

Concentration of Sucrose in sample

$$5.84 \text{ nmole}/50.0 \ \mu\text{L} = 0.117 \text{ nmole}/\mu\text{L}$$

$$0.117 \text{ nmole}/\mu\text{L} \times 342.3 \text{ ng/nmole} = 40.0 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	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 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 Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	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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