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Product Information

Total Carbohydrate Assay Kit

Catalog Number **MAK104**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
Carbohydrates are the most abundant biomolecules present in all living organisms. Carbohydrates have many functions, as structural components to the cell walls of bacteria and plants, or as energy storage in the form of starch and glycogen. Carbohydrates are also a major component of the human diet.

The Total Carbohydrate Assay Kit can be used for measuring carbohydrates in a variety of samples, including food and beverage products. Total carbohydrate concentration is based on the phenol-sulfuric acid method in which polysaccharides are hydrolyzed and then converted to furfural or hydroxylfurfural. These compounds react with the Developer to generate a chromagen, which can be detected spectrophotometrically at 490 nm.

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

Assay Buffer Catalog Number MAK104A	25 mL
Developer Catalog Number MAK104B	3 mL
D-Glucose Standard, 2 mg/mL Catalog Number MAK104C	0.2 mL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- Temperature controlled heat block
- Concentrated sulfuric acid (H_2SO_4, Catalog Number 258105)

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.

Preparation Instructions
Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents.

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

Developer – Developer is stable at room temperature while in use.

Storage/Stability
The kit is shipped on wet ice and storage at 2–8 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Glucose Standards for Colorimetric Detection
Add 0, 2, 4, 6, 8, and 10 µL of the 2 mg/mL standard solution directly into a 96 well plate, generating 0 (blank), 4, 8, 12, 16, and 20 µg/well standards. Add water to each well to bring the volume to 30 µL.

Sample Preparation
Tissue (50 mg) or cells (1×10^6) can be homogenized in 200 µL of ice-cold Assay Buffer. Centrifuge the samples at 13,000 $\times$ *g* for 5 minutes to remove insoluble material.

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

Bring samples to a final volume of 30 µL with water.

Assay Reaction
1. Add 150 µL of the concentrated sulfuric acid (H_2SO_4, not provided) to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 15 minutes at 90 °C. Cover the plate and protect from light during the incubation.

2. Add 30 µL of Developer to each well. Mix well using horizontal shaker for 5 minutes at room temperature.

3. Mix contents for 1 minute before measuring the absorbance at 490 nm (A_{490}).

Results

Calculations

The background for the assay is the value obtained for the 0 (blank) Glucose standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

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

The amount of total carbohydrate present in the samples may be determined from the standard curve.

Concentration of carbohydrate

$$S_a/S_v = C$$

S_a = Amount of carbohydrate in unknown sample (glucose equivalents) from standard curve
S_v = Sample volume (μL) added to reaction well
C = Concentration of carbohydrate in sample

Sample Calculation

Amount of carbohydrate (S_a) = 10.48 μg (from standard curve)
Sample volume (S_v) = 50 μL

Concentration of carbohydrate in sample

$$10.48\ \mu g/50\ \mu L = 0.2096\ \mu g/\mu L$$

Total carbohydrate concentration in samples may also be expressed in μg/μg of protein or mg/g of sample.

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 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 needed to use 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 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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