Technical Bulletin

Total Carbohydrate Assay Kit

Catalogue number MAK559

Product Description

Carbohydrates are the most abundant biomolecules present in all living organisms. Carbohydrates perform various key roles in living organisms- they are a fuel source (Glucose), they are used for energy storage (Glycogen and Starch), as a structural component (cellulose), as parts of proteins (glycoproteins, proteoglycans), as components of the genetic material (Ribose and Deoxyribose), for survival (Trehalose) and more.

The Total Carbohydrate Assay Kit can be used for measuring carbohydrates in a variety of samples, including food and beverage products, solutions, cell culture supernatant and cells. The total carbohydrate concentration assay is based on a method in which polysaccharides are hydrolyzed and then converted to an intermediate. This intermediate reacts with the developer to generate a chromogen, which can be detected spectrophotometrically at 490 nm.

Components

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

- Assay Buffer 25 mL
  Catalogue Number MAK559A
- Developer 3 mL
  Catalogue Number MAK559B
- D-Glucose Standard, 2 mg/ml 0.2 mL
  Catalogue Number MAK559C.

Reagents and Equipment Required but Not Provided

- Concentrated sulfuric acid, H_2SO_4.
- 96-well plates, clear, flat bottom. It is recommended to use clear plates for colorimetric assays.
- Plate reader that is capable to read wavelength of 490 nm.
- 90 °C oven
- Horizontal Shaker
- Vortex Mixer

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 product is shipped at room temperature. Store components at 2-8 °C upon receipt.

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.

Procedure

All Samples and Standards should be run in technical triplicates.

Preparation of Glucose Standards

1. 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.
2. Add ultrapure water to each well, to bring to a final volume of 30 µL.

Sample Preparation

Tissue or cells can be homogenized using ice-cold Assay Buffer.

1. 1 million cells should be resuspended in Assay Buffer at a final volume of 200 µL.
2. Centrifuge the samples at 13,000 g for 5 minutes to remove insoluble material.
3. Allow the assay buffer to lyse the cells for 5 minutes at room temperature. The final Sample volume in the well is 30 µL.

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.

Assay Reaction

1. After completing the incubation in the sample preparation step, add 30 µL of each sample to the clear, flat bottom 96-well plate with the standards. The volume in each well for standards and samples is 30 µL.

Note: All samples and standards should be run in technical triplicates. It is highly advisable to use a multi-channel pipettor and troughs, when possible, to minimize technical error.

2. Add 150 µL of the concentrated sulfuric acid (H_2SO_4, not provided) to each of the wells. It is highly advisable to use dispenser pipettor, if possible, to minimize the technical error.
3. Mix well using a horizontal shaker for 5 minutes and incubate the reaction for 15 minutes at 90 °C.

Measurement

1. Measure the absorbance at 490 nm (A490) using a plate reader. These values will serve as the blank value and will be subtracted from the final absorbance at 490nm, after the assay is performed.
2. Add 30 µL of Developer to each well. This step should be carried out in a chemical fume hood. It is highly advisable to use a multi-channel pipettor and a trough, to minimize the technical error.
3. Measure the absorbance at 490 nm (A490) again, using a plate reader. These values are the final absorbance for each well.

Results

Calculations

The background of each well is the value of A490 obtained from the first A490 reading (blank). 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 must be determined from the standard curve.

Concentration of Carbohydrates:

Sa/Sv = C

Sa = The amount of carbohydrate in the unknown sample (glucose equivalents), calculated from the standard curve

Sv = Sample volume (µL) added to the sample well

C = Concentration of total carbohydrates in the sample

Sample Calculation

Amount of carbohydrates (Sa) = 4.8 µg

(Calculated from the standard curve)

Sample volume (Sv) = 30 µL

Concentration of carbohydrate in sample, before dilution:

4.8 µg/30 µL = 0.16 µg/µL (or mg/mL)

Do not forget to multiply by the dilution factor to obtain the total carbohydrates concentration of the original, undiluted sample.

In favor of simplifying the calculations, a convenient and simple to use online excel-based calculator can be downloaded from the webpage of MAK559.

Figure 1:
An exemplary standard curve.

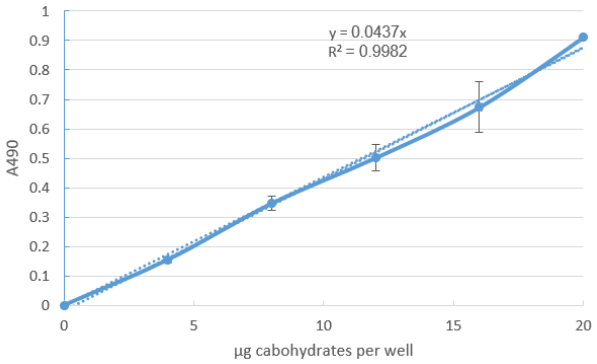


Figure 2:
An exemplary result of the assay when performed on common drinks.

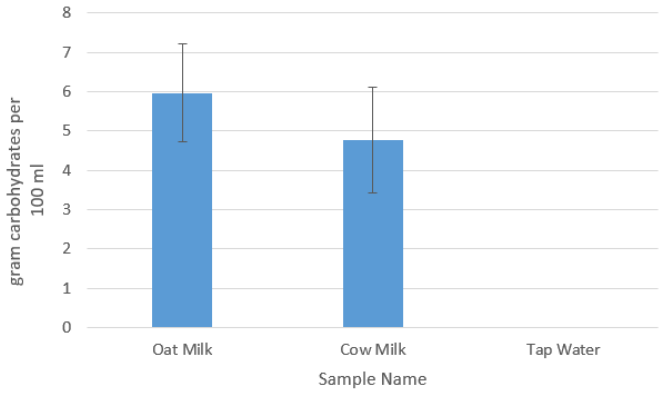
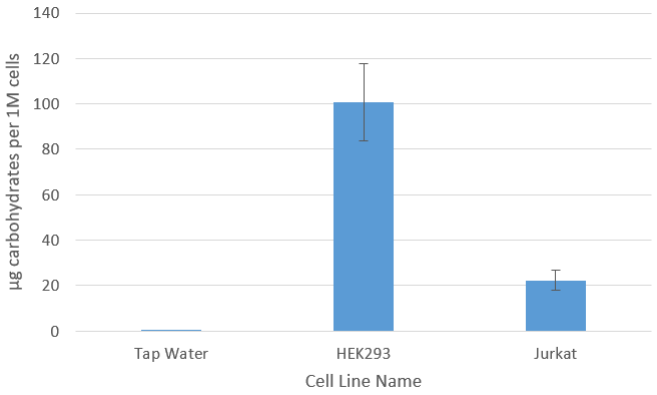


Figure 3:
An exemplary result of the assay when performed on common cell lines.



References

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2. Nielsen, Suzanne S.Food analysis laboratory manual. *springer,* ISBN 978-1-4419-1462-0 (2017).
3. Yue F, Zhang J *et.al.,* Effects of monosaccharide composition on quantitative analysis of total sugar content by phenol-sulfuric acid method. *Front Nutr*. Aug 2 (2022)
4. Jain VM, *et.al.,* Estimating the carbohydrate content of various forms of tobacco by phenol-sulfuric acid method. J *Educ Health Promot*. Oct 4 (2017)

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 flat bottom, 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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