MA-0144

Total Carbohydrate Assay

(100 wells, Colorimetric, OD 490 nm, Store at room temperature)

Introduction:

Carbohydrates are one of the fundamental building blocks of biochemistry. The total carbohydrate assay is a crucial analytical technique used to determine overall carbohydrate content in each sample. This assay provides valuable information for a wide range of applications in the food and beverage industries, pharmaceutical research, and basic biochemistry. The assay involves the oxidation of sugars to aldehydes which react with phenol to give color around 490 nm. Scientists utilize the assay to assess the nutritional value of food products, verify the efficiency of carbohydrate-based pharmaceutical formulations, and monitor carbohydrate metabolism in biological samples. AkrivisBio's Total Carbohydrate Assay's accuracy and reliability make it an invaluable tool in scientific research and commercial industries, contributing to improved product development and a deeper understanding of carbohydrate-related phenomena. Moreover, it serves as an essential tool in quality control processes, enabling manufacturers to ensure consistency and adherence to standards.

Assay Principle:

1 – Sulfuric acid dehydrates sugars with the formation of aldehydes
2 – The aldehydes react with phenol producing an absorbance band centered around 490 nm

Assay Components:

Assay Buffer	25 ml	WM	MA-0144A
Phenol Solution	3 ml	Amber bottle	MA-0144B
Glucose Standard	0.2 ml	Yellow	MA-0144C

User Supplied Reagents and Equipment:

- Concentrated H_2SO_4 (98%)
- Temperature controlled Heat Block

Note: This assay makes use of concentrated Sulfuric acid (H_2SO_4). This is a highly hazardous material, very corrosive and oxidizing. Handle only with all necessary protective clothing, goggles, gloves etc.

Storage and Handling:

Store all components at room temperature. All components are ready to use as supplied.

Assay Protocol:

1. Standard Curve: Transfer 0 – 5 – 10 – 15 – 20 – 25 µl of Glucose Standard to a series of wells in a 96-well plate, giving 0, 4, 8, 12, 16, 20 µg/well of Glucose Standard. Adjust all well volumes to 30 µl with DI H_2O.

2. Test Samples: Homogenize tissue (10 mg) or cells (1 x 10^6) with 200 µl of Assay Buffer. Centrifuge at 16,000 X g for 5 min. and transfer the clear supernatant to a fresh tube. Add 5-30 µl of sample per well. Liquid samples can be added directly to wells. Adjust all well volumes to 30 µl with H_2O.

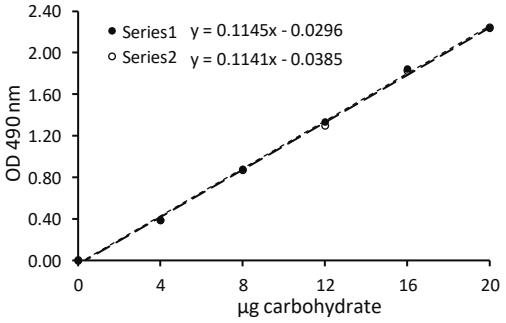
3. Initiate Reaction: Add 150 µl concentrated H_2SO_4 to all Standard and Test wells. Mix thoroughly on a shaker and incubate at 90°C for 15 min.

4. Color Development: After 15 min, add 30 µl of Phenol Solution. Place on a shaker for 5 min at room temperature.

5. Measurement: measure OD at 490 nm in endpoint mode.

6. Typical Results:

µg Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.0605	0.0671	0.0000	0.0000
4	0.4482	0.4522	0.3877	0.3851
8	0.9393	0.9339	0.8788	0.8668
12	1.3992	1.3591	1.3387	1.2920
16	1.9105	1.8914	1.8500	1.8243
20	2.2967	2.3131	2.2363	2.2460



7. Calculation: Subtract the zero Standard value from all other standards and test sample readings. Plot the Glucose Standard Curve. Determine the slope of the standard curve. The slope defines the sensitivity of the assay system. Divide the background corrected test sample values by the slope of the standard curve. This converts the OD to µg of carbohydrate in the wells. To convert this to µg carbohydrate in the original samples:

A – Divide the µg carbohydrate in the well by the volume of sample added to the well = µg carbohydrate per µl of sample.

B – Multiply the µg carbohydrate per µl of sample x total volume of supernatant transferred in step 2 above = total µg carbohydrate in sample.

C – Divide the total µg carbohydrate in sample by the mg tissue used or # of cells used = µg of carbohydrate per mg tissue (or per # of cells, etc.)

FOR RESEARCH USE ONLY! Not to be used on humans.