ab155891

Total Carbohydrate Quantification Assay Kit

Instructions for Use

For the sensitive and accurate measurement of Total Carbohydrate in various samples

View kit datasheet: www.abcam.com/ab155891
(use www.abcam.cn/ab155891 for China, or www.abcam.co.jp/ab155891 for Japan)

This product is for research use only and is not intended for diagnostic use.

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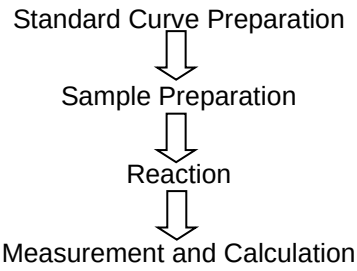
1. Overview

Carbohydrates play important structural as well as chemical roles in all living systems. Detection of total carbohydrates, therefore, has wide applications. Abcam's Total Carbohydrate Assay is a simple, sensitive and robust method of detecting virtually all carbohydrates. The assay is based on the phenol-sulfuric acid method.

In Abcam's Total Carbohydrate Assay, polysaccharides (mono, di, tri, etc.) and their derivatives, in the presence of sulfuric acid, are hydrolyzed to monomers and converted to furfural or hydroxyfurfural, which react with the Developer Solution IV to form a chromogen that can be quantified by measuring the absorbance at 490 nm. The Total Carbohydrate Assay can detect most forms of carbohydrates, including simple and complex saccharides, glycans, glycoproteins and glycolipids.

2. Protocol Summary



3. Components and Storage

All components in this kit are shipped on blue ice and are suitable for storage at -20ºC, unless reconstituted. Upon receipt, immediately store kit at -20ºC in the dark. Individual components may be stored at alternative temperatures as show in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

A. Kit Components

Item	Quantity	Storage Temperature
Assay Buffer 7	25 mL	-20°C
Developer Solution IV	3 mL	-20°C or +4°C
D-Glucose Standard	0.2 mL	-20°C or +4°C

PLEASE NOTE: Assay Buffer 7 was previously labelled as Assay Buffer VII and Assay Buffer. The composition has not changed.

B. Additional Materials Required

- Concentrated H_2SO_4 (98%)
- 96-well clear plate with flat bottom
- Temperature controlled Heat Block
- Multi-well spectrophotometer (plate reader)

- Safety goggles and gloves

Caution: H_2SO_4 is highly corrosive and oxidizing; handle with protective clothing, goggles and gloves etc. Do not add water to concentrated acid.

4. Assay Protocol

Carbohydrate Assay Protocol

1. **Glucose standard curve**:
   Add 0, 2, 4, 6, 8 and 10 µl of D-Glucose Standard into a series of wells of 96-well microtiter plate to generate 0, 4, 8, 12, 16 and 20 µg/well of D-Glucose Standard. Adjust volume to 30 µl per well with dH_2O.

2. **Sample Preparation:**
   Liquid samples can be measured directly. Homogenize tissue (50 mg) or cells (1 x 10^6) with 200 µl ice cold Assay Buffer 7. Centrifuge at 12000 rpm for 5 minutes. Collect the supernatant. Add 1-30 µl of sample per well and adjust the volume to 30 µl with dH_2O.

Note: For unknown samples, we suggest testing several doses of samples to ensure the readings are within the Standard Curve range.

3. **Reaction:**
   Add 150 µl concentrated H_2SO_4 (98%, not provided) to D-Glucose Standard and sample wells, mix for one min on a shaker and

incubate at 90°C for 15 min. After 15 min, add 30 µl Developer Solution IV. Mix on shaker for 5 min at room temperature.

4. Measurement:

Mix the contents for 1 min. and measure OD at 490 nm.

5. Data Analysis

Calculation: Subtract the 0 Standard from all readings. Plot the Glucose Standard Curve. Apply the sample OD to the Standard Curve to get B µg of total carbohydrate (glucose equivalent) amount.

$$\textbf{Total carbohydrate concentration} = \frac{\textbf{B}}{\textbf{V}} \times \textbf{Dilution Factor} = \textbf{µg/µL or mg/mL}$$

Where:

B is the amount of total carbohydrate from Standard Curve (glucose equivalents).

V is the sample volume added into the reaction well (µl).

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

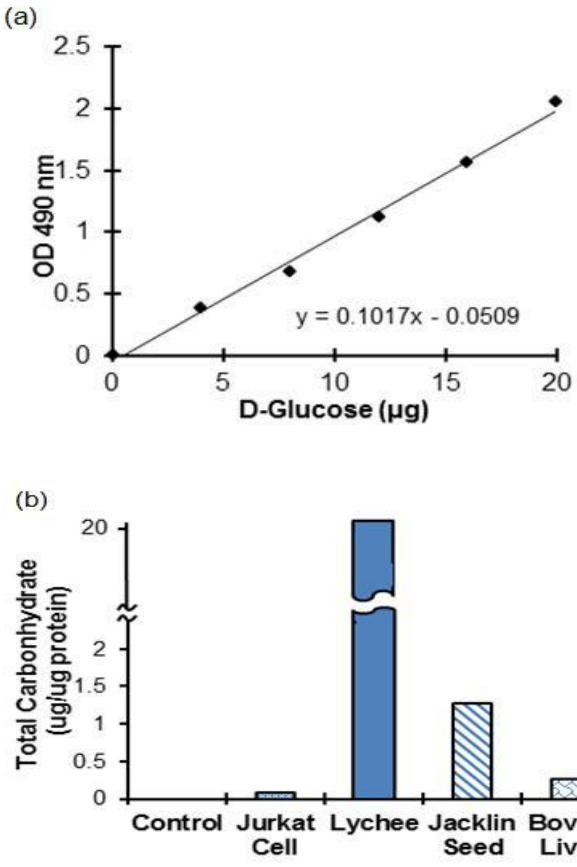


Figure. D-Glucose Standard curve **(a)** Total carbohydrate concentration in jurkat cell lysate, lychee, jacklin seed and bovine liver respectively **(b).** Assays were performed following kit protocol.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the Assay Buffer 7 provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

 For all technical or commercial enquiries please go to:
www.abcam.com/contactus
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www.abcam.co.jp/contactus (Japan)