RayBio® Total Carbohydrate Assay Kit

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RayBio® Total Carbohydrate Assay Kit Protocol

(Cat#:68-Carb-S100)

RayBiotech, Inc.

We Provide You With Excellent Support And Service

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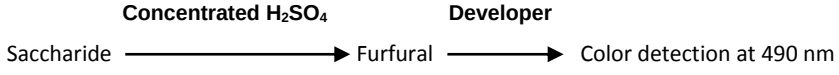
I. INTRODUCTION

Carbohydrates play important structural as well as chemical roles in all living systems. Detection of total carbohydrates, therefore, has wide applications. RayBiotech'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 our 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 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.



II. APPLICATION

- Measurement of total carbohydrate in various samples.

III. SAMPLE TYPE

- Adherent or cells in suspension
- Animal and plant tissues
- Food products, fruit juices & other beverages

IV. KIT CONTENTS

Store Kit at 4°C

Components	Size	Cap Code	Part Number
Assay Buffer	25 ml	WM	Item A
Developer	3 ml	Brown Bottle	Item B
Standard (D-Glucose, 2 mg/ml)	0.2 ml	Yellow	Item C

V. USER SUPPLIED REAGENTS AND EQUIPMENT

- 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.

VI. STORAGE AND HANDING

Warm Assay Buffer to room temperature before use. Developer is stable at room temperature. Kit components are stable at 4°C for up to a year if stored properly.

VII. CARBOHYDRATE ASSAY PROTOCOL

1. **Glucose Standard Curve:** Add 0, 2, 4, 6, 8 and 10 µl of Glucose Standard into a series of wells of 96-well microtiter plate to generate 0, 4, 8, 12, 16 and 20 µg/well of 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 (1×10^6) with 200 µl ice cold Assay Buffer. 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 dilutions 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 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. Mix on shaker for 5 min at room temperature.

4. **Measurement:** Mix the contents for 1 min. and measure OD at 490 nm.

5. **Calculation:** Subtract 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.

Total carbohydrate concentration in the sample = B/V x Dilution Factor = 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.

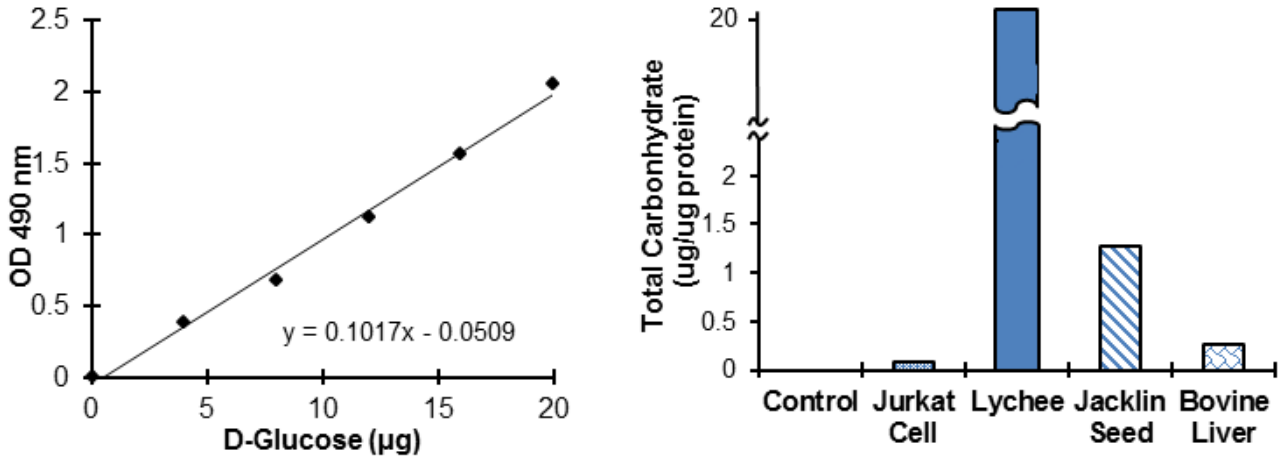


Figure. D-Glucose Standard Curve (a). Total carbohydrate concentration in jurkat cell lysate, lychee, jacklin seed & bovine liver respectively (b). Assays were performed following kit protocol.

This product is for research use only.