Product Manual

Total Carbohydrate Assay Kit

Catalog Number

STA-682 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Carbohydrates are molecules made up of oxygen, hydrogen, and carbon. Typically, carbohydrates contain a hydrogen: oxygen atom ratio of 2:1 (as with water). In the field of biochemistry, carbohydrates are viewed synonymously with saccharides, which describe sugars, starch, and cellulose. The saccharides can be further categorized as monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Typically, the lower molecular weight monosaccharides (such as glucose) and disaccharides (such as sucrose) are referred to as sugars. Carbohydrates perform key roles in living organisms such as storing energy, as parts of coenzymes, as part of genetic material (DNA and RNA) as well as structural roles.

The measurement of total carbohydrate concentration is important in several fields such as the food industry, pharmaceutical research, petroleum industry, as well as environmental research. Many techniques such as light scattering, nuclear magnetic resonance, capillary electrophoresis, infrared spectroscopy, and chromatography have been used to detect total carbohydrates, but these techniques are costly, time consuming, and require complex analytical skills.

Cell Biolabs' Total Carbohydrate Assay Kit is a simple colorimetric assay that measures the amount of total carbohydrate present in foods, urine, plasma, serum, tissue homogenates, or cell lysates in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, standards and unknown samples. Sample carbohydrate concentrations are determined by comparison with a known glucose standard. The kit has a detection sensitivity limit of 62.5 µM glucose.

Assay Principle

Cell Biolabs' Total Carbohydrate Assay Kit measures total carbohydrate within food samples, urine, serum, plasma, lysate, or tissue samples based on the phenol-sulfuric acid method. Carbohydrates are hydrolyzed to furfural and other derivative forms in the presence of sulfuric acid. Upon addition of Developing Solution, a chromogen is formed that can be detected at 490 nm (Figure 1).

Figure 1. Total Carbohydrate Assay principle.

Related Products

1. STA-398: Free Glycerol Assay Kit (Colorimetric)
2. STA-399: Free Glycerol Assay Kit (Fluorimetric)
3. STA-672: S-Adenosylmethionine (SAM) ELISA Kit

4. STA-674: Glutamate Assay Kit
5. STA-675: Hydroxyproline Assay Kit
6. STA-680: Glucose Assay Kit (Colorimetric)
7. STA-681: Glucose Assay Kit (Fluorometric)

Kit Components (shipped at room temperature)

1. 10X Assay Buffer (Part No. 268201): One 15 mL bottle.
2. 100X Diluent (Part No. 268204): One 1.0 mL tube.
3. Glucose Standard (Part No. 268203): One 200 µL tube at 40 mM

Materials Not Supplied

1. Concentrated Sulfuric Acid (18 M)
2. Phenol
3. Temperature controlled heat block
4. Orbital shaker
5. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
6. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
7. Standard 96-well clear microtiter plate and/or clear cell culture microplate
8. Multichannel micropipette reservoir
9. Spectrophotometric microplate reader capable of reading at OD 490 nm

Storage

Store Glucose Standard at 4°C and store the other components at room temperature.

Preparation of Reagents

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity.
- 1X Diluent: Dilute the stock 100X Diluent 1:100 with deionized water for a 1X solution. Stir or vortex to homogeneity.
- Developing Solution: Dilute Phenol to 5% final concentration in 1X Diluent. Vortex to mix.

Preparation of Samples

- Cell culture supernatants: Cell culture media containing glucose or other added carbohydrates should be avoided. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The cell

conditioned media can be assayed directly or diluted as necessary. Prepare the Glucose standard curve in the same non-conditioned media without carbohydrates.

- Tissue lysates: Sonicate or homogenize tissue sample in cold PBS or 1X Assay Buffer and centrifuge at 10000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates can be assayed undiluted or diluted as necessary in 1X Assay Buffer.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant can be assayed directly or diluted as necessary in 1X Assay Buffer.

Note: All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.

Preparation of Standard Curve

Prepare fresh Glucose standards before use by diluting in 1X Assay Buffer. Use the 40 mM Glucose Standard to prepare a dilution series according to Table 1 below

Standard Tubes	40 mM Glucose Solution (µL)	1X Assay Buffer (µL)	Glucose (mM)
1	20	180	4
2	100 of Tube #1	100	2
3	100 of Tube #2	100	1
4	100 of Tube #3	100	0.5
5	100 of Tube #4	100	0.25
6	100 of Tube #5	100	0.125
7	100 of Tube #6	100	0.063
8	0	100	0

Table 1. Preparation of Glucose Standards.

Assay Protocol

Note: Sulfuric acid is highly corrosive and can damage certain types of plastics. Avoid using plastics that are sensitive to sulfuric acid such as polystyrene, and test plastics prior to attempting this assay by adding 100 uL of sulfuric acid and heating to 90ºC for 10-15 minutes. Sulfuric acid should be handled with care. Gloves, a lab coat, and protective eyewear should be worn during handling. Sulfuric acid should be stored in glassware only and be pipetted in a fume hood.

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 30 µL of each glucose standard or unknown sample into a microcentrifuge tube or a clear 96-well plate.

3. Add 150 µL of concentrated sulfuric acid to each tube or well. Incubate for 15 minutes at 90ºC.
4. Transfer to 4°C for 2-3 minutes.
5. If samples are in tubes, carefully transfer each sample to a 96-well plate.
6. Read samples at OD 490 nm to determine background.
7. Mix the Developing Solution by vortex and immediately add 30 µL to each well. Mix on an orbital shaker for 5 minutes.
8. Read samples at OD 490 nm to determine signal.
9. Subtract background OD (step 6) from signal OD (step 8).

Example of Results

The following figures demonstrate typical Total Carbohydrate Assay Kit (Colorimetric) results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

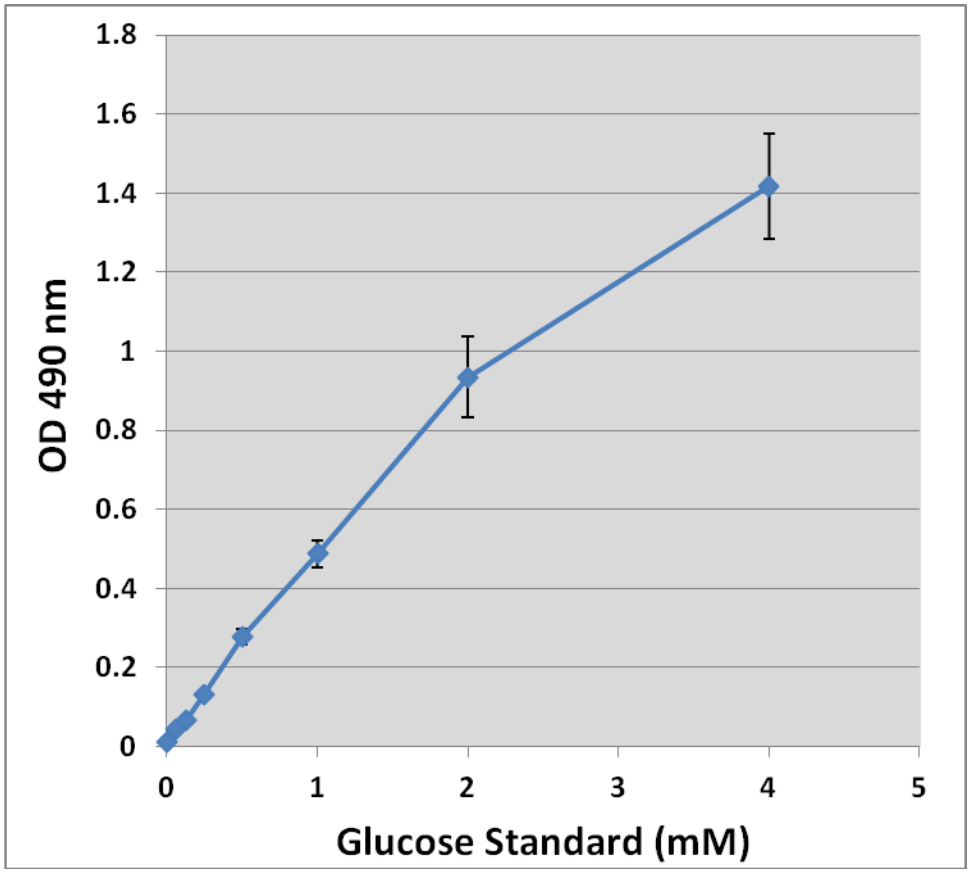

Figure 2: Glucose standard curve.

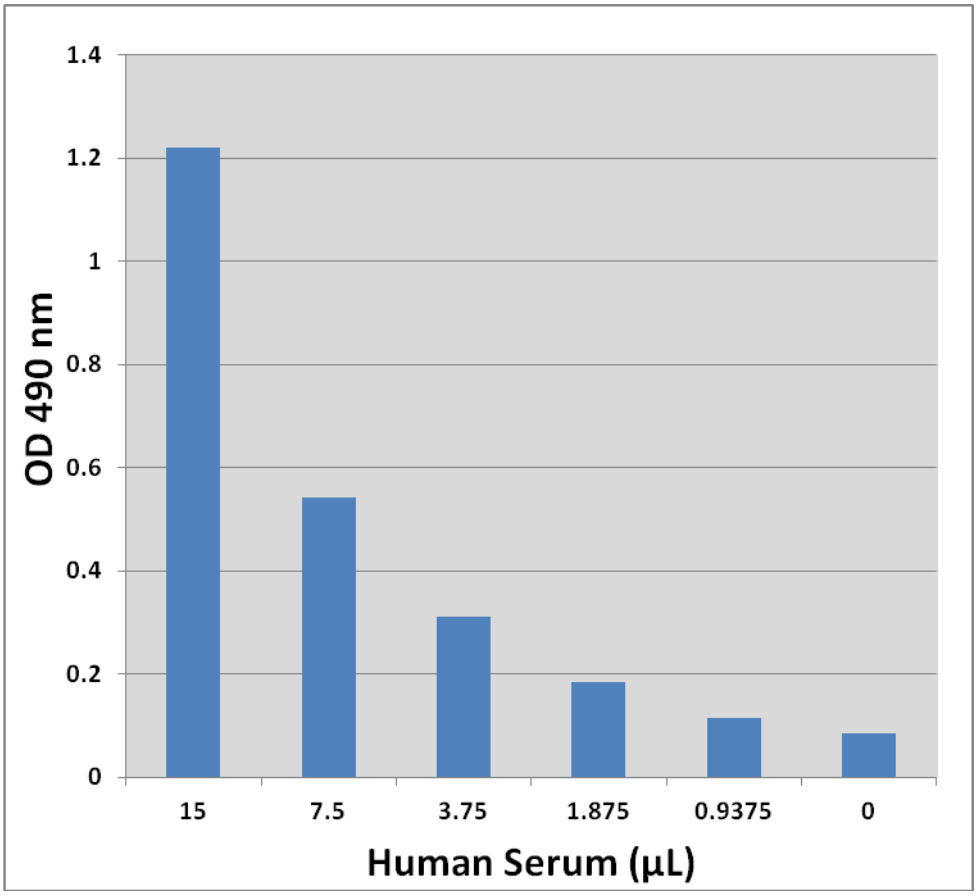

Figure 3: Total carbohydrate detection in human serum using Total Carbohydrate Assay Kit.

References

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Recent Product Citations

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Warranty

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