Datasheet for ABIN5067617

Total Carbohydrate Assay Kit

3 Images

Go to Product page

Overview

Quantity:	100 tests
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	Total Carbohydrate Assay Kit measures total carbohydrate within food samples, urine, serum, plasma, lysate, or tissue samples based on the phenol-sulfuric acid method.
Sensitivity:	62.5 µM
Characteristics:	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.
Components:	1. 10X Assay Buffer : One 15 mL bottle. 2. 100X Diluent : One 1.0 mL tube. 3. Glucose Standard : One 200 µL tube at 40 mM

Target Details

Background:	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

Target Details

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.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Protocol:	Carbohydrates are hydrolyzed to furfural and other derivative forms in the presence of sulfuric acid. Upon addition of Developing Solution, a chromagen is formed that can be detected at 490 nm .
Reagent Preparation:	• 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity.
Sample Preparation:	• 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 3 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 106 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.
Assay Procedure:	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 µLof sulfuric acid and heating to 90 °C 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

Application Details

fume hood.

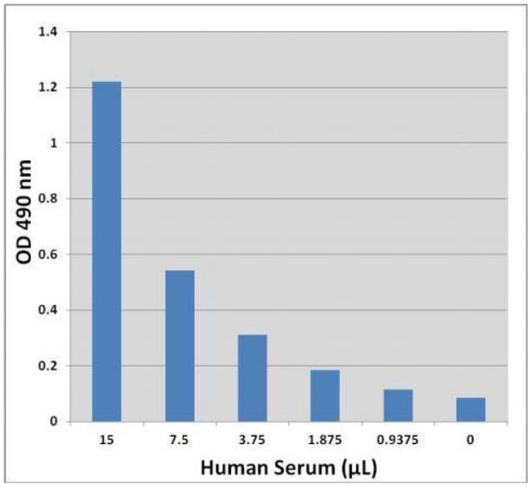
1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 30 µL of each glucose standard or unknown sample into a microcentrifuge tube or a clear 96- well plate. 4
3. Add 150 µL of concentrated sulfuric acid to each tube or well. Incubate for 15 minutes at 90 °C.
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. Add 30 µL of Developing Solution and mix on an orbital shaker for 5 minutes. Note: Developing Solution contains a diluted form of phenol. Avoid inhalation or contact with skin.
8. Read samples at OD 490 nm to determine signal.
9. Subtract background OD (step 6) from signal OD (step 8).

Restrictions:	For Research Use only

Handling

Storage:	RT/4 °C
Storage Comment:	Store Glucose Standard at 4°C and store the other components at room temperature.

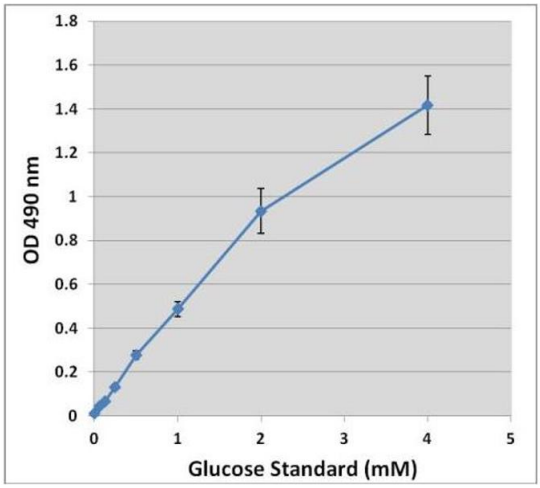
Images



Biochemical Assay

Image 1. Total carbohydrate detection in human serum using Total Carbohydrate Assay Kit.

Image 2. Total Carbohydrate Assay principle.



Biochemical Assay

Image 3. Glucose standard curve