Carbohydrate (total) Assay Kit

Carbohydrate (total) Assay Kit is a detection kit for the quantification of Carbohydrate (total) in serum, plasma, urine, cell culture supernatants, cell lysate and tissue lysate.

Catalog number: ARG82228

Package: 100 assays

For research use only. Not for use in diagnostic procedures.

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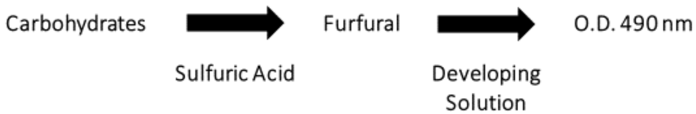
INTRODUCTION

A carbohydrate is a biomolecule consisting of carbon (C), hydrogen (H) and oxygen (O) atoms, usually with a hydrogen–oxygen atom ratio of 2:1 (as in water) and thus with the empirical formula $C_m(H_2O)_n$ (where m may or may not be different from n). However, not all carbohydrates conform to this precise stoichiometric definition (e.g., uronic acids, deoxy-sugars such as fucose), nor are all chemicals that do conform to this definition automatically classified as carbohydrates (e.g. formaldehyde and acetic acid).

Carbohydrates perform numerous roles in living organisms. Polysaccharides serve for the storage of energy (e.g. starch and glycogen) and as structural components (e.g. cellulose in plants and chitin in arthropods). The 5-carbon monosaccharide ribose is an important component of coenzymes (e.g. ATP, FAD and NAD) and the backbone of the genetic molecule known as RNA. The related deoxyribose is a component of DNA. Saccharides and their derivatives include many other important biomolecules that play key roles in the immune system, fertilization, preventing pathogenesis, blood clotting, and development. [Provide by Wikipedia: Carbohydrate]

PRINCIPLE OF THE ASSAY

This Carbohydrate (total) Assay Kit employs a simple colorimetric assay to measure the total amount of carbohydrate in foods, urine, plasma, serum, tissue homogenates, or cell lysates. The assay is 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 chromagen is formed that can be detected at 490 nm. The concentration of carbohydrates in the samples is then determined by comparing the 490 nm absorbance of samples to the standard curve.



MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Glucose Standards (40 mM)	200 µL	4°C
10X Assay Buffer	15 mL	Room temperature
100X Diluent Buffer	1 mL	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate Reader capable of reading at O.D. 490 nm
- Standard 96-well clear microtiter plate or clear cell culture microplate
- Microplate shaker
- Concentrated Sulfuric Acid (18M)
- Phenol
- Deionized or Distilled water
- Temperature controlled heat block
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, Store Glucose Standard at 4°C and store the other components at room temperature.
- 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 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 fume hood.
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatant: Cell culture media containing glucose or other added carbohydrates should be avoided. To remove insoluble particles, centrifuge at 2500 x g for 10 minutes. 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 10,000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay buffer.

Cell Lysates: Re-suspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge at 10,000 x g for 10 minutes at 4°C to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in 1X Assay Buffer.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g and 4ºC for 10 minutes. The supernatant can be assayed directly or diluted as necessary in 1X Assay Buffer.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. The supernatant can be assayed directly or diluted as necessary in 1X Assay Buffer.

Urine: To remove insoluble particles, centrifuge at 2500 x g for 10 minutes. The supernatant can be assayed directly or diluted as necessary in 1X Assay Buffer.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum / plasma sample.
3. 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.

REAGENT PREPARATION

- **1X Assay buffer:** Dilute the 10X Assay Buffer into deionized water to yield 1X Assay Buffer. (E.g., add 5 mL of 10X Assay Buffer into 45 mL of deionized water to a final volume of 50 mL) Stir or vortex to homogeneity.
- **1X Diluent Buffer:** Dilute the 100X Diluent Buffer into deionized water to yield 1X Diluent Buffer. (E.g., add 100 µL of 100X Diluent Buffer into 9.9 mL of deionized water to a final volume of 10 mL) Stir or vortex to homogeneity.
- **Developing Solution:** Dilute Phenol to 5% final concentration in 1X Diluent Buffer. Vortex to mix.

- **Standards:** Prepare fresh Glucose Standards by diluting in 1X Assay Buffer according to the Table below.

Standard tubes	Final Glucose conc. (mM)	Volume of 1X Assay Buffer (µL)	Volume of Glucose Standard (µL)
S1	4	180	20 of 40 mM Glucose Standard
S2	2	100	100 of S1
S3	1	100	100 of S2
S4	0.5	100	100 of S3
S5	0.25	100	100 of S4
S6	0.125	100	100 of S5
S7	0.063	100	100 of S6
S0	0	100	0

ASSAY PROCEDURE

Prepare and mix all reagents thoroughly before use. Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

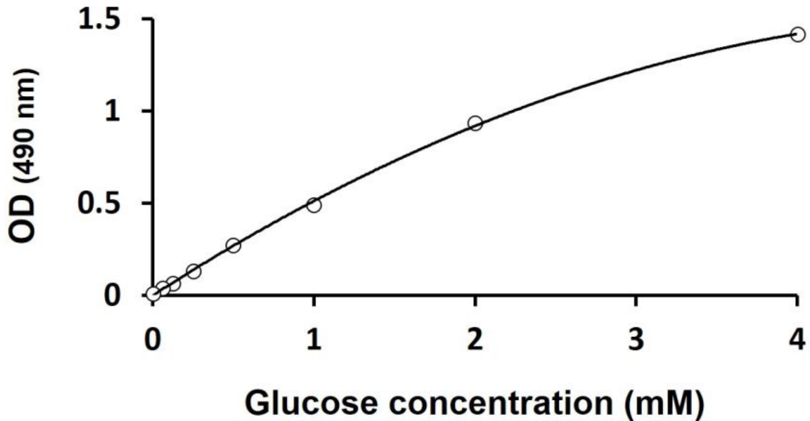
1. Add **30 µL** of **samples** or serial **diluted Glucose Standards** into 96-well microplate.
2. Add **150 µL** of **concentrated sulfuric acid** to each well.
3. Incubate for **15 minutes** at **90°C**.
4. Stand for **2-3 minutes** at **4°C**.
5. Read the plate at **490 nm** using a microplate reader to determine background.
6. Mix the **Developing Solution** by vortex and immediately add **30 µL** to each well. Mix on a microplate shaker for **5 minutes**.
7. Read the plate at **490 nm** using a microplate reader to determine signal.
8. Subtract background O.D. (step 5) from signal O.D. (step 7)

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of standards and samples.
2. Subtract the average value of Standard 0 from all standard values.
3. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
4. The concentration of carbohydrates in the samples is then determined by comparing the 490 nm absorbance of samples to the standard curve.

EXAMPLE OF TYPICAL STANDARD CURVE

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



QUALITY ASSURANCE

Sensitivity

0.063 mM

Intra-assay and Inter-assay precision

The CV value of intra-assay and inter-assay was ≤ 10 %