

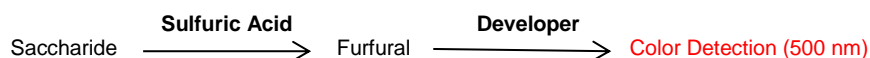
High Sensitivity Carbohydrate Assay Kit (Colorimetric)

06/20

(Catalog # K2049-100; 100 assays; Store at 4°C)

I. Introduction:

Carbohydrates are one of the most important components in many foods and the most abundant biomolecules present in all living organisms. Quantitation of total carbohydrate content is an essential analytical procedure in many fields including food and beverage products, agricultural products, medicinal products, vaccine pharmaceuticals etc. **BioVision's High Sensitivity Carbohydrate Assay Kit** is a simple, sensitive, robust assay for the quantification of carbohydrates even when present in low concentration. The assay can be used for the quantitative determination of any carbohydrates that contain neutral sugars and uronic acids. The reaction is a modification of phenol-sulfuric acid method. In this assay, carbohydrates are hydrolyzed and converted to hydroxyfurfural and furfural, which then react with a highly sensitive developer to form a colored complex, detected spectrophotometrically at 500 nm.



II. Application:

- Quantitation of total carbohydrate in vaccines and various samples.

III. Sample Types:

- Vaccines and biologicals
- Adherent or suspension cells
- Animal and plant tissues
- Food products such as fruit juices & other beverages

IV. Kit Contents:

Components	K2049-100	Cap Code	Part Number
Carbohydrate Developer	1.5 ml	Amber	K2049-100-1
Standard (D-Glucose, 2 mg/ml)	0.2 ml	Yellow	K2049-100-2

V. User Supplied Reagents and Equipment:

- PBS
 - Conc. H₂SO₄ (98%)
 - dH₂O
 - 96-well clear plate with flat bottom
 - Multi-well spectrophotometer (plate reader)
 - Safety goggles and gloves
- Caution:** H₂SO₄ is highly corrosive and oxidizing. Handle with protective clothing's, goggles, gloves etc. Do not add water to the concentrated acid.

VI. Storage Conditions and Reagent Preparation:

Store the kit at 4°C. Read the entire protocol before performing the assay.

- **Carbohydrate Developer:** Ready to use. Store at 4°C. Bring to room temperature (RT) before performing the assay.
- **Standard (D-Glucose, 2 mg/ml):** Ready to use. Store at 4°C. Bring to RT before performing the assay.

VII. High Sensitivity Carbohydrate Assay Protocol:

1. **Sample Preparation:** Liquid samples can be measured directly after removing any insoluble particles by centrifugation. **Tissue or Cell lysates:** Homogenize tissues (50 mg) or cells (1 x 10⁶ cells) in 200 µl ice cold PBS. Centrifuge at 12,000 x g and 4°C for 10 min. Collect the supernatant (lysate) into a fresh new tube for the assay. Add 2-30 µl of Sample(s) into designated well(s) of a 96-well clear plate. Adjust the volume to 30 µl/well with dH₂O.

Notes:

- a. We recommend using freshly prepared samples for analysis. Store the samples(s) at -80°C for future experiments.
 - b. For Unknown Samples, we suggest testing several doses of samples to ensure that the readings are within the Standard Curve range.
2. **Glucose Standard Curve:** Add 0, 2, 4, 6, 8 and 10 µl of Glucose Standard into a series of a 96-well clear plate to generate 0, 4, 8, 12, 16 and 20 µg/well of Glucose Standard. Adjust the volume to 30 µl/well with dH₂O.
 3. **Reaction:** Add 150 µl conc. H₂SO₄ to Standard and Sample(s) wells and mix at RT for one min on a shaker. Then add 10 µl Carbohydrate Developer to all the wells. Mix well again at RT for one min on a shaker and incubate at 90°C for 15 min. After 15 min, allow the plate to cool down at RT for 5 min on a shaker.

Note:

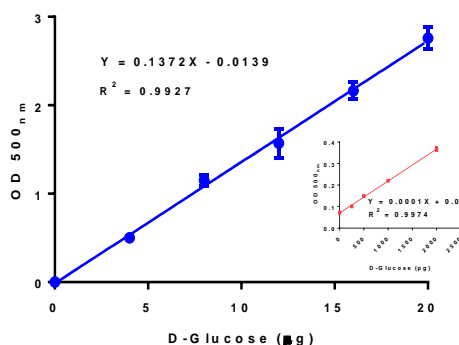
- a. Carbohydrate Developer is viscous. Thus, mix well after adding by pipetting.

4. **Measurement:** Measure the absorbance of all wells at 500 nm.
5. **Calculation:** Subtract 0 Standard from all Standard and Sample readings. Plot the Glucose Standard Curve. Apply the corrected Sample readings to the Glucose Standard Curve to get B µg of total carbohydrate (glucose equivalent) in the sample.

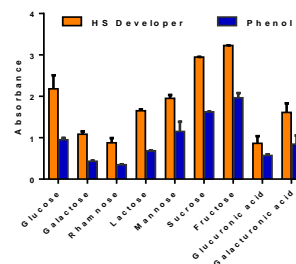
$$\text{Total Carbohydrate Concentration (C) in Sample(s) wells} = \frac{B}{V} \times D = \mu\text{g}/\mu\text{l or mg/ml}$$

Where, **B** = Amount of total carbohydrate from the Glucose Standard Curve (glucose equivalent)
V = Volume of sample added per well (μl)
D = Sample dilution factor ($D = 1$ for undiluted samples)

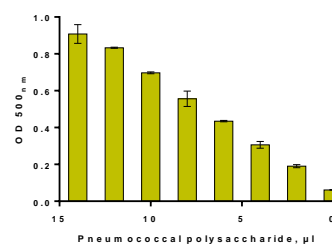
(a)



(b)



(c)



Figures: (a). D-Glucose Standard Curve. (b). Absorbance of Monosaccharides upon reacting with High Sensitivity (HS) Carbohydrate Developer and Phenol. (c). Total Polysaccharide content in Pneumococcal Polysaccharide Powder Type 4 (US Type 4), calculated total carbohydrate concentration (glucose equivalent) is $0.47 \pm 0.02 \mu\text{g}/\mu\text{l}$. The assay was performed according to the kit assay protocol.

VIII. Related Products:

Total Carbohydrate Colorimetric Assay Kit (Cat. # K645-100)
 Glucose Colorimetric/Fluorometric Assay Kit (Cat. # K606-100)
 EZScreen™ Glucose Colorimetric Assay Kit (384 Well) (Cat. # K950-384)
 Glucose Colorimetric Assay Kit II (Cat. # K686-100)
 PicoProbe™ Glucose Fluorometric Assay Kit (Cat. # K688-100)
 Glucose and Sucrose Colorimetric/Fluorometric Assay Kit (Cat. # K616-100)
 Maltose and Glucose Colorimetric/Fluorometric Assay Kit (Cat. # K618-100)

FOR RESEARCH USE ONLY! Not to be used on humans.