ab284543 – High Sensitivity Carbohydrate Assay Kit (Colorimetric)

For the quantitation of total carbohydrate in vaccines and various samples
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

http://www.abcam.com/ab284543

Storage and Stability

On receipt entire assay kit should be stored at 4°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Carbohydrate Developer	1.5 mL	4°C
Standard (D-Glucose, 2 mg/ml)	0.2 ml	4°C

Materials Required, Not Supplied

- 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.

Reagent Preparation

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

Assay Protocol

Sample preparation:

1. Liquid samples can be measured directly after removing any insoluble particles by centrifugation.
2. **Tissue or Cell lysates:** Homogenize tissues (50 mg) or cells (1 x 10^6 cells) in 200 µL ice cold PBS. Centrifuge at 12,000 x g and 4°C for 10 min.
3. Collect the supernatant (lysate) into a fresh new tube for the assay.
4. Add 2-30 µL of Sample(s) into designated well(s) of a 96-well clear plate.
5. Adjust the volume to 30 µL/well with dH2O.

Δ 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.*

Standard Curve Generation

1. 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.
2. Adjust the volume to 30 µL/well with dH2O.

Reaction Mix Preparation

1. 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.
2. Mix well again at RT for one min on a shaker and incubate at 90°C for 15 min.
3. After 15 min, allow the plate to cool down at RT for 5 min on a shaker.

Δ Note: *Carbohydrate Developer is viscous. Thus, mix well after adding by pipetting.*

Measurement

Measure the absorbance of all wells at 500 nm.

Calculation

1. Subtract 0 Standard from all Standard and Sample readings.
2. Plot the Glucose Standard Curve.
3. Apply the corrected Sample readings to the Glucose Standard Curve to get B µg of total carbohydrate (glucose equivalent) in the sample.

Total Carbohydrate Concentration (C) in Sample(s) wells = B/V x D = µg/µL 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)

Technical Support

For all technical or commercial enquiries please go to:
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www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)