HiPer® Carbohydrates Estimation Teaching Kit (Quantitative)

Product Code: HTBC003

Number of experiments that can be performed: 10

Duration of Experiment

Protocol

- DNSA Method :1 hour
- Phenol Sulphuric Acid Method: 1 hour

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store all the kit contents as specified in the brochure

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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Aim:

To determine the concentration of carbohydrates by two commonly used methods

1. **3, 5- Dinitrosalicylic Acid (DNSA) Method**
2. **Phenol Sulphuric Acid Method**

Introduction:

Carbohydrates are the most abundant class of organic compounds found in living organisms. Carbohydrates are a major source of metabolic energy, both for plants and for animals and they also serve as a structural material (cellulose), a component of the energy transport compound ATP, recognition sites on cell surfaces, and one of three essential components of DNA and RNA.

Carbohydrates are classified according to their molecular size and solubility. Carbohydrates are classified into groups according to the number of individual simple sugar units:

1) **Monosaccharides:** They are the simplest form of sugar and are usually colorless, water soluble and crystalline solids, e.g. glucose, fructose, galactose, etc.
2) **Disaccharides:** It is formed when two monosaccharides undergo condensation reaction and are water soluble, e.g. sucrose, lactose, etc.
3) **Polysaccharides:** It is formed when more than two monosaccharides units bound together by glycosidic bonds, e.g. starch, glycogen, cellulose, etc.

In order to measure the concentration of carbohydrates present in a solution, either DNSA or Phenol Sulphuric Acid method can be followed.

Materials Required But Not Provided:

Glasswares: Pipettes (1 ml and 10 ml), Cuvettes, Test tubes, Glass beakers/Bottles

Reagents: Distilled water*, Sulphuric acid (Concentrated), Phenol Solution (5%)

Other requirements: Spectrophotometer/Colorimeter to determine the absorbance in given range, Micropipette and tips, Test tube stand

*Molecular biology grade water is recommended (Product code: ML064)

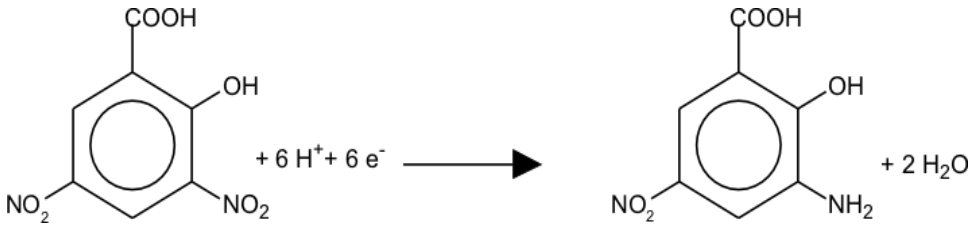
Storage:

HiPer® Carbohydrates Estimation Teaching Kit (Quantitative) can be stored at RT for up to 12 months from date of manufacture without showing any reduction in performance. Read Important Instructions before starting the experiment. Store all the reagents as specified in the brochure.

1. DNSA Method:

Principle:

3, 5-Dinitrosalicylic acid (DNSA) is used extensively in biochemistry for the estimation of reducing sugars. It detects the presence of free carbonyl group (C=O) of reducing sugars. This involves the oxidation of the aldehyde functional group (in glucose) and the ketone functional group (in fructose). During this reaction DNSA is reduced to 3- amino- 5-nitrosalicylic acid (ANSA) which under alkaline conditions is converted to a reddish brown coloured complex which has an absorbance maximum of 540 nm.



3, 5 Dinitrosalicylic acid (DNSA) **3- amino- 5 nitrosalicylic acid (ANSA)**

Fig 1: Chemical Reaction for DNSA Method

Kit Contents:

Table 1: Enlists the materials provided in this kit for DNSA method with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			10 expts	
1	TKC239	Glucose Standard (1 mg/ml)	7.2 ml	2-8^{O}C
2	TKC240	DNSA Reagent	50 ml	RT
3	GRM598	Potassium sodium tartrate, tetrahydrate	20 g	RT
4	TKC241	Test Sample 1 (for DNSA Method)	2.4 ml	2-8^{O}C
5	TKC242	Test Sample 2 (for DNSA Method)	2.4 ml	2-8^{O}C

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. Always use dry and clean glasswares.
3. The unknown and standard samples should be treated identically for accurate results.
4. The assay should be carried out at the same time and in the same buffer conditions.
5. Sugar test samples provided are of different concentrations (Test Sample 1 and 2).
6. **Preparation of 40% Potassium sodium tartrate (Rochelle's salt) (50 ml):** Dissolve 20 g of Potassium sodium tartrate in 25 ml of distilled water* and make up the volume to 50 ml.

***Recommended product for use: ML064 – Molecular Biology Grade Water**

Procedure:

1. Take eight tubes and label them as Blank and 1 to 7.
2. Make dilutions of glucose standards with concentrations of 40, 80, 120, 160 and 200 µg per 200 µl by transferring respective amount of glucose from the standard glucose solution (1mg/ml) and adjusting it to a total volume of 200 µl by adding distilled water as mentioned in Table 2.
3. Add 0.5 ml of DNSA reagent to all the eight test tubes. Mix well.
4. Keep in boiling water bath for 15 minutes.
5. Add 0.5 ml of 40% Potassium sodium tartrate (Rochell's Salt) solution and mix it well.
6. Switch on the spectrophotometer and select the wavelength of 540 nm. First take the absorbance (OD) of Blank and make it zero.
7. Take the OD of all the tubes (No. 1-7). Wash the cuvettes each time after taking OD.

Table 2:

Tube No.	**Blank**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Conc. Of Glucose (µg)	0.0	40	80	120	160	200	Test sample 1	Test Sample 2
Vol. of Glucose std. taken (µl)	0.0	40	80	120	160	200	200 µl	200 µl
Vol. of distilled water added (µl)	200	160	120	80	40	0.0		
Vol. of DNSA added (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Keep in boiling water bath for 15 minutes								
Vol. of 40 % Rochell's Salt added	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Absorbance at 540 nm								

8. Plot a standard curve of absorbance at 540 nm on "Y" axis versus concentration of glucose in µg/200 µl on "X" axis
9. Record the value "x" of unknown from graph corresponding to the OD reading of the test samples.

Determination of Concentration of Test Sample:

Sugar concentration can be calculated using the following formula:

Sugar Concentration in Test Sample = Concentration of unknown "x" in µg/200 µl

= …… x 5 µg/ml

Observation and Result:

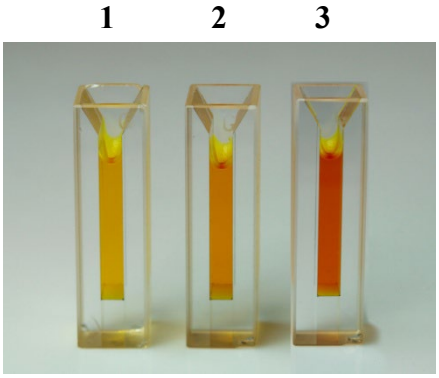
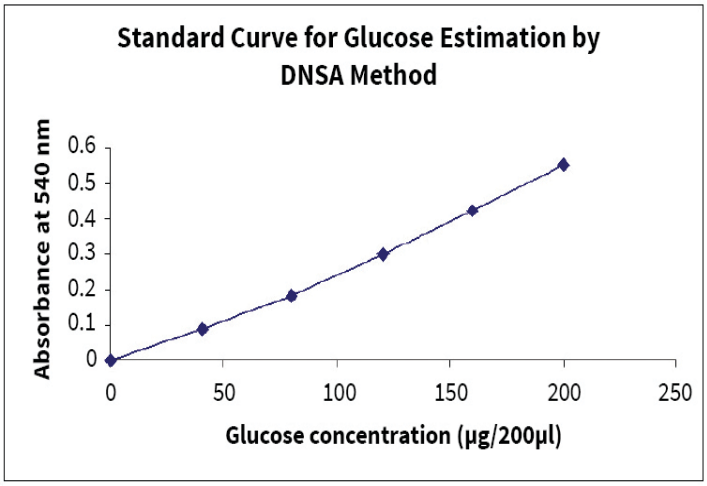


Fig 2: Carbohydrate Estimation by DNSA Method - showing increasing amount of sugar concentration



The absorbance of glucose solution at 540 nm increases with increasing concentration of glucose

Interpretation:

The DNSA method is carried out by preparing a set of solutions with known glucose concentrations and mixing them with the DNSA reagent. A standard curve is plotted and the concentrations of unknown sugar samples can be derived from the standard curve.

2. Phenol Sulphuric Acid Method:

Principle:

Phenol - Sulphuric Acid Method is the most easiest and reliable method amongst the quantitative assays for carbohydrate estimation. It is mostly used in measuring neutral sugar content in oligosaccharides, proteoglycans, glycoproteins and glycolipids.
In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This forms a yellow-brown coloured product with phenol and has absorption maximum at 490 nm. This is one of the best methods to estimate total carbohydrate.

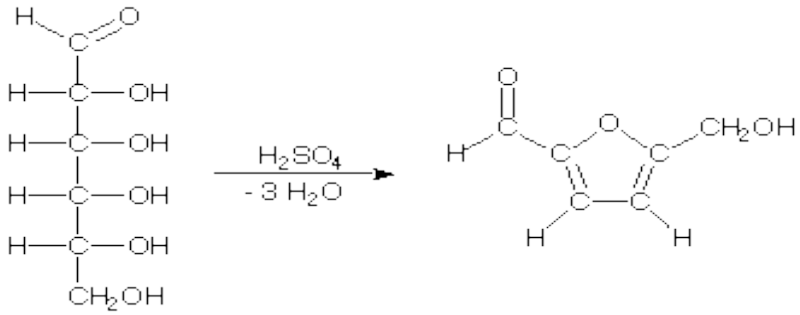


Fig 3: Chemical Reaction in Phenol Sulphuric acid Method

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. Always use dry and clean glasswares.
3. The unknown and standard samples should be treated identically for accurate results.
4. The assay should be carried out at the same time and in the same buffer conditions.
5. Sugar test samples provided are of different concentrations (Test Sample 1 and 2).
6. Before starting the experiment set a water bath at 25 ° – 30°C.

Kit Contents:

Table 3: Enlists the materials provided in this kit for Phenol Sulphuric Acid method with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			10 expts	
1	TKC239	Glucose Standard (1 mg/ml)	7.2 ml	2-8°C
2	TKC248	Test Sample 1 (Phenol Sulphuric Acid method)	2.4 ml	2-8°C
3	TKC249	Test Sample 2 (Phenol Sulphuric Acid method)	2.4 ml	2-8°C

Procedure:

1. Take eight tubes and label them as Blank and 1-7.
2. Make dilutions of glucose standards with concentrations of 40, 80, 120, 160 and 200 µg/200µl by transferring respective amount of glucose from the standard glucose solution (1mg/ml) and adjusting it to a total volume of 200 µl by adding distilled water as mentioned in Table 3.
3. Add 0.2 ml of 5 % phenol solution to all the tubes.
4. Add 1 ml of concentrated Sulphuric acid to each tube and mix it well.
5. After 10 minutes, mix the contents of the tubes and place in a water bath set at 25-30^{o}C for 20 minutes.
6. Switch on the Spectrophotometer, select the wavelength of 490 nm. First take the absorbance (OD) of Blank and make it zero.
7. Take the OD of all the tubes (1 – 7). Wash the cuvette each time after taking OD.

Table 3:

Tube No.	**Blank**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Conc. of Glucose (µg)	0.0	40	80	120	160	200	Test sample 1	Test Sample 2
Volume of Glucose stock taken (µl)	0.0	40	80	120	160	200	200 µl	200 µl
Volume of distilled water added (µl)	200	160	120	80	40	0		
Volume of 5 % Phenol solution added (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Volume of conc. Sulphuric acid added (ml)	1	1	1	1	1	1	1	1
Keep at room temperature for 10 minutes, Mix well and place in a water bath at 25 – 30^{0}C for 20 minutes								
Absorbance at 490 nm								

8. Plot a standard curve of absorbance at 490 nm on "Y" axis versus concentration of glucose in µg/200µl on "X" axis
9. Record the value "x" of Unknown from graph corresponding to the OD reading of the test samples.

Determination of Sugar Concentration in Unknown Sample:

Sugar concentration can be calculated using following formula:

Sugar Concentration in Test Sample = Concentration of unknown "x" in µg/200 µl

= ……. x 5 µg/ml

Observation and Result:

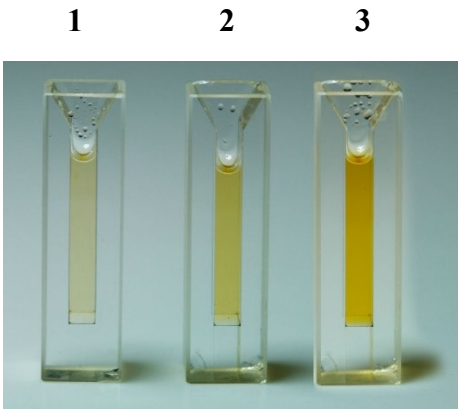
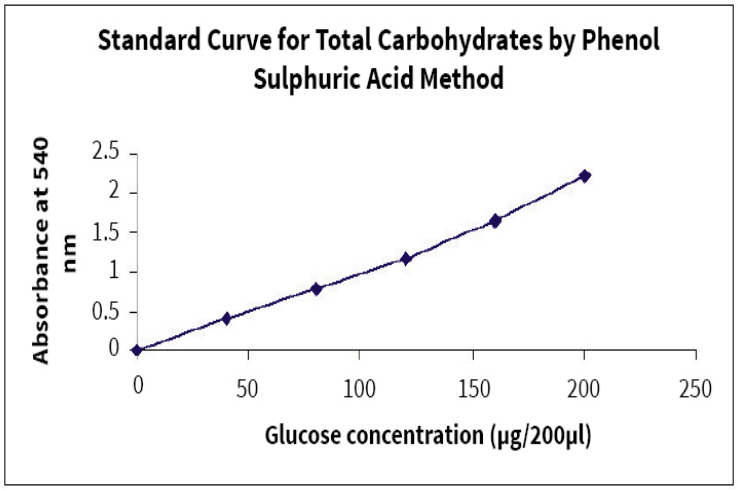


Fig 4: Carbohydrate Estimation by Phenol Sulphuric Acid Method - showing increasing amount of sugar concentration



The absorbance of the glucose solution at 490 nm increases with increasing concentration of glucose

Interpretation:

The Phenol Sulphuric Acid method is carried out by preparing a set of solutions with known glucose concentrations and mixing them with the Phenol- Sulphuric acid reagent. A standard curve can be made and the concentrations of unknown sugar samples can be derived from the standard curve.

Trouble shooting Guide:

Sr. No	Problem	Possible Cause	Solution
1	Standard and test samples give lower OD values than expected although the Blank is ok	Procedure was not carried out properly	Follow the entire procedure carefully
		Absorbance was not measured at correct wavelength	Measure absorbance at correct wavelength as mentioned in the brochure

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of Technical assistance mail at mb@himedialabs.com

Symbol:

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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