HiPer® Carbohydrates Estimation Teaching Kit (Qualitative)

Product Code: HTBC002

Number of experiments that can be performed: 25

Duration of Experiment: 4 hours

Storage Instructions:

- This kit is stable for 6 months from the date of receipt
- This kit can be stored at room temperature (15-25ºC)

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Aim:

To identify and characterize the different types of carbohydrates by commonly used qualitative methods:

1. **Molisch's Test**
2. **Benedict's Test**
3. **Barfoed's test**
4. **Seliwanoff's Test**
5. **Bial's Test**
6. **Iodine Test**

Introduction:

Carbohydrates are organic molecules which contain carbon, hydrogen and oxygen and are the most abundant class of organic compounds found in living organisms. It is the main energy source for animals as they breakdown carbohydrates during the process of metabolism to release energy. Carbohydrates 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.

All carbohydrates are made up of units of sugar or saccharide units. They 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:** Carbohydrates that contain only one sugar unit are called monosaccharides. These are the simplest form of sugar and are usually colourless, water soluble and crystalline solids, e.g. glucose, fructose, galactose etc.
2) **Dissacharides:** Carbohydrates that contain two sugar units are called disaccharides. It is formed when two monosaccharides undergo condensation reaction and are water soluble, e.g. sucrose, lactose etc.
3) **Polysaccharides:** Carbohydrates that contain more than two sugar units are called disaccharides. It is formed when more than two monosaccharide units bound together by glycosidic bonds, e.g. starch, glycogen, cellulose etc.

Principle:

Presence of carbohydrates can be confirmed qualitatively by several tests. Due to the presence of different numbers of sugar units specific carbohydrates exhibit typical colour reactions that form the basis for their identification. In this kit the following tests will be performed for the qualitative analysis of carbohydrates:

1. Molisch's Test:

All carbohydrates (monosaccharides, disaccharides and polysaccharides) give a positive reaction for Molisch test. It is based on the dehydration of the carbohydrate by Sulphuric acid to produce an aldehyde, which condenses with two molecules of α-naphthol, resulting in appearance of a purple ring at the interface.

Molisch's Reaction

2. Benedict's test:

Benedict's test is based upon the participation of the aldehyde and ketone groups in a chemical reaction. In this procedure, Benedict's reagent, which contains a basic solution of cupric ions, is mixed with a sugar. The copper ions will attract the electrons from the aldehyde or ketone group changing the charged copper ions to a neutral metal. Copper ions are blue in color whereas copper metal is orange. Disaccharides will react with the Benedict's reagent only if it contains an exposed aldehyde or ketone group. This type of sugar is known as a reducing sugar.

Benedict's Reaction

3. Barfoed's Test

The Barfoed's test is used to distinguish between reducing monosaccharides and disaccharides. Barfoed's reagent contains cupric ions in an acidic solution. Milder conditions and careful control of the heating time allow monosaccharides, but not disaccharides, to be oxidized. The cupric ions are reduced in the presence of a monosaccharide resulting in a red precipitate.

Barfoed's Reaction

4. Seliwanoff's Test:

Seliwanoff's test is used to distinguish between ketoses and aldoses. If a sugar contains a ketone group it is called ketose and if it contains an aldehyde group then it is called aldose. Ketoses are more rapidly dehydrated than aldoses when heated. The dehydrated ketose, hydroxymethyl furfural, then reacts with the resorcinol to produce a deep cherry red color.

Seliwanoff's Reaction

5. Bial's Test:

By performing Bial's test one can distinguish between a pentose and hexose. Bial's reagent contains orcinol in concentrated HCl with a small amount of $FeCl_3$ catalyst. In presence of concentrated HCl pentoses form furfural and hexoses form 5-hydroxymethyl. Furfural reacts with orcinol and $FeCl_3$ to produce a blue-green product. Hexoses give 5-hydroxyfurfural upon dehydration and 5-hydroxyfurfural reacts with Bial's reagent to give a brownish coloured product.

Bial's Reaction

6. Iodine Test:

Iodine forms colored complexes with polysaccharides. The color of the complex depends upon the three dimensional structure of the polysaccharide. Starch is a coiled structure which turns blue when bound to Iodine whereas glycogen, which is a branched molecule, turns red-violet.

Kit Contents:

The kit can be used to perform qualitative analysis of carbohydrates.

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

Sr. No.	Product Code	Materials Provided	Quantity 25 expts	Storage
1	RM077	Standard Sugar 1(Glucose)	1.1 g	RT
2	RM3029	Standard Sugar 2 (Starch)	0.55 g	RT
3	RM196	Standard Sugar 3 (Fructose)	0.05 g	RT
4	RM197	Standard Sugar 4 (Ribose)	0.3 g	RT
5	TKC243	Test Sugar 1	2 g	R T
6	TKC244	Test Sugar 2	2 g	R T
7	TKC245	Test Sugar 3	2 g	RT
8	RM389	1-naphthol	3.75 g	R T
9	R002	Benedict's Reagent	2X260 ml	R T
10	TKC247	Barfoed's Reagent	220 ml	R T
11	RM153	Resorcinol	0.11 g	R T
12	RM460	Orcinol	0.81 g	RT
13	TKC250	Ferric chloride solution	1 ml	RT
14	S013	Iodine Reagent	22 ml	RT

Materials Required But Not Provided:

Glass wares: Test tubes, Beakers

Reagents: Distilled water, Concentrated Sulphuric acid, Concentrated Hydrochloric acid, Ethanol 99%

Other requirements: Test tube holder, Test Tube stand, Bunsen burner, Boiling water Bath, Pipettes/Dropper, Bottles for preparation of solutions

Storage:

HiPer® Carbohydrates Estimation Teaching Kit (Qualitative) is stable for 6 months from the date of receipt without showing any reduction in performance. Store the entire kit contents at room temperature (15-25°C).

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. Always use dry and clean glasswares.
3. Test sugars and Standard sugars are provided separately.

   NOTE: Please refer Table 2 of Page 7 for Standard Sugars to be used.
4. **Preparation of Standard Sugars:**
   - Standard Sugar 1 (Glucose) – Dissolve 1.1 g of powder in 110 ml of water, mix well to dissolve.
   - Standard Sugar 2 (Starch) – Dissolve 550 mg of powder in 55 ml of water, mix well to dissolve.
   - Standard Sugar 3 (Fructose) – Dissolve 50 mg of powder in 5 ml of water, mix well to dissolve.
   - Standard Sugar 4 (Ribose) – Dissolve 300 mg of powder in 30 ml of water, mix well to dissolve.
5. **Preparation of Test Sugars:**
   - Test Sugar 1 – Dissolve 2 g of powder in 200 ml of water, mix well to dissolve.
   - Test Sugar 2 – Dissolve 2 g of powder in 200 ml of water, mix well to dissolve.
   - Test Sugar 3 – Dissolve 2 g of powder in 200 ml of water, mix well to dissolve.

   NOTE: All the Test and Standard sugar solutions should be stored at 2 – 8 °C.
6. **Preparation of Bial's Reagent (20 ml):** Dissolve 810 mg of orcinol in 110 ml of concentrated HCl, add 0.675 ml of Ferric chloride solution and dilute to 270 ml with distilled water. The reagent is stable at RT.
7. **Preparation of Seliwanoff's Reagent (20 ml):** Dissolve 110 mg of Resorcinol in 220 ml of 3N HCl. The reagent is stable at RT.
8. **Preparation of Molisch's Reagent :** Dissolve 3.75 g of 1-naphthol in 25 ml of Ethanol 99%.

Procedure:

Read the important instructions before starting the experiment.

NOTE: Take 4 test tubes and label it as Standard, Test sugar 1, Test sugar 2 and Test sugar 3 individually for all the below mentioned tests.

A) Molisch's Test for Carbohydrates:

1. Take 2 ml of each Standard and test sugar solutions in four test tubes separately.
2. Add 2 drops of Molisch reagent to each tube.
3. Separately pour these solutions slowly into four tubes each containing 2 ml of concentrated sulphuric acid so that two layers will form. Look for the purple ring at the interface.

B) Benedict's Test for Reducing Sugars:

1. Add 1 ml of Standard and Test solutions to 5 ml of Benedict's reagent separately and shake each tube.
2. Mix well and heat the tubes in boiling water bath for 3 minutes and allow them to cool down. Look for red/yellow precipitate.

C) Barfoed's Test for Reducing Monosaccharides:

1. Take 1 ml of each Standard and Test solutions in four test tubes.
2. Add 2 ml of Barfoed's reagent to each tube and heat in a boiling water bath for 3 minutes. Look for brick red precipitate.

D) Seliwanoff's Test for Ketoses:

1. Take 0.5 ml of Standard and Test sugar solutions in four test tubes.
2. Add 2 ml of Seliwanoff's reagent to each tube and heat in a boiling water bath for 2 minutes. Look for the formation of red colour.

E) Bials' Test for Pentoses:

1. Take 1 ml of Standard and Test sugar solutions in four test tubes
2. Add 2.5 ml of Bial's reagent to each tube and heat in a boiling water bath for 1 minute and allow the tubes to cool down to room temperature. Look for the formation of blue-green colour.

F) Iodine Test for Starch:

1. Take 2 ml of each Standard and Test samples in four test tubes.
2. Heat the solution of the tubes for 1 minute.
3. Add four drops of Iodine reagent to each tube. Look for the formation of a blue-black product.

Observation and Result:

Table 2: After performing each test, record the results of the test sugars as in the following table

Sr No.	Test	Standard Sugars to be used for +ve results	Test Sugar 1	Test Sugar 2	Test Sugar 3
1	Molisch's Test	Standard Sugar 1 (Glucose)			
2	Benedict's Test	Standard Sugar 1 (Glucose)			
3	Barfoed's test	Standard Sugar 1 (Glucose)			
4	Seliwanoff's Test	Standard Sugar 3 (Fructose)			
5	Bial's Test	Standard Sugar 4 (Ribose)			
6	Iodine Test	Standard Sugar 2 (Starch)			

PositiveTest is indicated by:

1. **Molisch's Test:** The formation of a purple product at the interface of two layers
2. **Benedict's Test:** Precipitation of copper oxide as a red, yellow, or yellowish-green solid
3. **Barfoed's Test:** The formation of brick red precipitate
4. **Seliwanoff's Test:** The formation of a red product
5. **Bial's Test:** The formation of a blue green product. All other colors indicate a negative result for pentoses.

   NOTE: Hexoses generally react to form green, red or brown products.
6. **Iodine Test:** The formation of a blue – black product

A B

Molisch's Test

A B

Benedict's Test

A B

Barfoed's Test

A B

Seliwanoff's Test

A B

Bial's Test

A B

Iodine Test

A: Negative Control

B: Positive Test

Interpretation:

Different types of carbohydrates are identified and characterized by using various qualitative methods of carbohydrates estimation.

1. **Molisch's Test:** Positive result indicates the presence of carbohydrate
2. **Benedict's Test:** Positive result shows the presence of reducing sugar
3. **Barfoed's Test:** Positive result shows the presence of reducing monosaccharide
4. **Seliwanoff's Test:** Positive result shows the presence of ketoses
5. **Bial's Test:** Positive result shows the presence of pentoses
5. **Iodine Test:** Positive result shows the presence of starch

Trouble shooting Guide:

Sr.No.	Problem	Possible Cause	Solution
1	False Positive or False Negative results	Procedure was not carried out properly	Follow the entire procedure carefully

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