

Organic Lab Protocol

Materials

10 test tubes
Test-tube rack
Test-tube holder
Pipets
Hot plate or water bath
600-mL beaker
Indicators:
Brown paper bag
Sudan III
Biuret reagent
Iodine (IKI)
Benedict's

5 mL distilled water
5 mL honey solution
5 mL egg white solution
5 mL corn oil
5 mL lettuce solution
5 mL gelatin solution
5 mL butter
5 mL potato solution
5 mL apple juice solution
5 mL unknown substance
Paper towels
Wash tub,
bottle brush
and soap



Safety

Use safety goggles. Be careful to avoid breakage when working with glassware. Always use special caution when using any laboratory chemicals, as they may irritate the skin or cause staining of the skin or clothing. Never touch or taste any chemical unless instructed to do so. Use extreme care when working with heated equipment or materials to avoid burns. Clean lab station completely and remove any residue from lab samples and indicators from table tops. Wash all equipment thoroughly and return it to the proper area. Wash your hands before leaving class.

Set up

Begin by filling your beaker to the 250mL mark with tap water. Place beaker on the hot plate on high until it boils and then turn the dial down to just keep the water hot. You will use this water bath for testing with Benedict's solution. Later on in lab, you will carefully pour the hot water into the cold soapy water in your wash tub to clean your glassware at the end of the lab experience. **CAUTION:** Hot water will cause severe burns if you are not careful. Always use the hot pads and be aware of your surroundings at all times. Keep all paper and plastic items away from hot plate at all times as they could catch on fire.

Part A. Testing for Lipids

1. Obtain 10 test tubes and place them in a test tube rack. Use black sharpie marker to number each test tube 1-10 at the top of each test tube.
2. Fill each test tube with 5 mL of the substances 1-10 from the stock solutions.
3. First use a ruler and divide your brown paper bag into 10 equal sections. In each section, write the number of the test tube sample. Use your finger to dab a little sample of each test tube on the appropriate square of the brown paper bag. Dry your finger between each sample to insure there is no cross contamination of samples. Set aside and let paper dry. Observe before the end of class and record your findings on your data table.
4. *Next,* Add 5 drops of Sudan III stain to each test tube sample. Sudan III stain will turn **red** in the presence of lipids.
5. Gently shake the contents of each test tube. **CAUTION:** *Sudan III will stain hands and clothing.* Record color changes in your Data Table and place a check mark next to substances testing positive for lipids.
6. Wash and rinse the test tubes thoroughly.

| | | | | |
|---|---|---|---|----|
| 1 | 2 | 3 | 4 | 5 |
| 6 | 7 | 8 | 9 | 10 |

Part B. Testing for Carbohydrates

Sugars and starches

1. Refill each clean test tube with 5 mL of the substances 1-10 from the stock solutions and return to your table.
2. Add 5 drops of iodine solution to each test tube. Iodine will change color from yellow-brown to blue-black in the presence of starch.
3. Gently shake the contents of each test tube. **CAUTION:** *Iodine is poisonous and can also stain hands and clothing.* In the Data Table, record any color changes and place a check mark next to those substances testing positive for **starch**.
4. Wash the test tubes thoroughly.



5. Next, refill each clean test tube with 5 mL of the substances 1-10 from the stock solutions and return to your table.
6. Add 10 drops of Benedict's solution to each test tube. Gently shake the contents of each test tube in a twirling motion of the wrist, one test tube at a time. **CAUTION:** *Benedict's solution may stain hands and/or clothing.*
7. Gently place test tubes in the hot water bath for about 3-5 minutes. **CAUTION:** Do not drop the test tube in the beaker as this will cause glassware to break.



When heated, Benedict's solution will change color from blue to yellow, orange, or red in the presence of a simple sugar/ monosaccharide (reducing sugar). *The green color represents a non-reducing sugar and needs to be tested using an additional method.

8. With the test tube holder, remove the test tubes from the hot-water bath and place them back in the test tube rack. **CAUTION:** *Always use a test tube holder to handle hot test tubes.*
9. Record color changes in your data table and place a check mark next to any substances that test positive for a simple sugar.
10. Wash the test tubes thoroughly.



Part C. Testing for Proteins

1. Refill each clean test tube with 5 mL of the substances 1-10 from the stock solutions and return to your table.
2. Add 5 drops of Biuret reagent to each test tube. **CAUTION:** *Biuret reagent contains a strong base, sodium hydroxide, and will cause skin irritation. If you splash any reagent on yourself, wash it off immediately with water and notify teacher.*
3. Gently shake the contents of each test tube in a twirling motion of the wrist, one test tube at a time. Biuret reagent changes color from yellow to blue-violet in the presence of protein.
4. Record color changes in your data table and place a check mark next to any substances that test positive for protein.
5. Wash test tubes thoroughly.



Clean lab tables and return all equipment to its original place. All lab tables should be **clean and dry** before students leave the lab setting. Please make sure you drain the wash tub by turning it upside down in the sink. Throw away all paper trash.