



Taking Macromolecules to Micro MiniLab

Student Guide

Cat# M3014

Version 061824



Name: _____

Date: _____

Worksheet 1 : Pre-Lab Questions:

1. What is a negative experimental control in a chemical test? Name an example of good negative control.

2. What are the four classes of biological macromolecules?

3. Complete the table below by filling in the missing information:

Macromolecule	Building block	Examples	Functions in living organisms
Carbohydrates			
Lipids			
	Amino acids		
	Nucleotides		

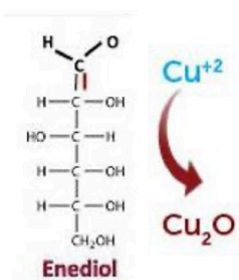
Test Reagent Guide

Benedict's Solution for Detecting Reducing Sugars:






This is a deep-blue alkaline solution used to test for the presence of simple carbohydrates, especially to identify reducing sugars, which have free ketone or aldehyde functional groups. When Benedict's solution and simple carbohydrates (such as glucose) are mixed together and heated, the copper (II) ions in the Benedict's solution are reduced to Copper (I) oxide, the solution changes color to green, yellow, orange or brick red with precipitates. Table 1 shows the relationship of the color formation and the amount of reducing sugar that is present in the solution.

Table 1: Benedict's Test Result

Color Observed	Reducing Sugar %	Result Interpretation
Blue	negative	no reducing sugar
Green with precipitation	~0.5 to 1.0%	Traceable (+)
Yellow with precipitation	~1.0 to 1.5%	Low (++)
Orange with precipitation	~1.5 to 2.0%	Moderate (+++)
Brick red with precipitation	> 2.0%	High (++++)



Benedict's Test Color Changes

				
Blue	Green ppt	Yellow ppt	Orange ppt	Brick red ppt
No reducing sugar	(Traceable ~0.5 to 1%)	(Low) ~1 to 1.5%	(Moderate) ~1.5 to 2%	(High) >2%

Iodine Solution for Detecting Starch:

This is a brown solution and used as an indicator for the presence of polysaccharides, primarily starches. Iodine reacts with starch and turns a blue/black color.

Biuret Reagent for Detecting Proteins:

The reagent is composed of sodium hydroxide, hydrated copper (II) sulfate and potassium sodium tartrate and is used to test for the presence of a peptide bond in substances, such as peptides, dipeptides or proteins. Under alkaline conditions, the blue-colored copper II ion forms a purple-colored complex with the peptide bonds, and turns the solution to purple. The deeper the purple color, the higher is the number of the peptide-copper complexes. The intensity of the purple color is directly proportional to the number of the peptide bonds or the number of the protein molecules present in the system.

GelGreen DNA Stain for Detecting DNA:

This is a compound that can bind to double-stranded DNA. It is non-fluorescent in solution. When bound to double-stranded DNA, it glows fluorescent green when illuminated with blue light. In this way, GelGreen is used as an indicator to show the presence of double stranded DNA in the solution.

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Worksheet 2: Basic Testing

There are five test stations in this lab activities. Each station has materials for testing one specific biological macromolecule. Each student group will take turns to go to each station to perform the tests and record the results in data table 2.

Station 1 - Lipid Testing: Oil

A. Material

- Lipid Test Card
- Bottle of Oil Solution
- Bottle of DI Water (Control)
- Paper towel for cleaning
- Fine point permanent marker

B. Procedure

1. Take an Oil Test card and label with your group name.
2. Drop one drop (~25 μL per drop) of the **Oil solution** onto the center of the circle labeled as "Oil".
3. Drop one drop (~25 μL per drop) of the **DI Water** onto the center of the circle labeled as "Water". This is the negative control. This is the negative control.
4. Allow the drops to sit and air dry (takes at least 10 minutes).
5. Compare and record results after finishing the other tests.

Station 2 - Carbohydrate Testing: Glucose

A. Material

- 6-well white test tray (one tray per student group; each group uses their tray for three tests)
- Bottle of Benedict's Solution
- Bottle of DI Water (Control)
- Bottle of Glucose Solution
- Paper towel for cleaning
- Fine point permanent marker
- Microwave oven

B. Procedure

1. Set the 6-well white test tray on the table.
2. Label a clean well from the test tray "G". Label a second clean well "W". See picture on assay station cards provided.
3. Drop two drops (~25 μL per drop) of the **Glucose Solution** to the well "G".
4. Drop two drops (~25 μL per drop) of the **DI Water** to the well "W".
5. Drop two drops (~25 μL per drop) of the **Benedict's Solution** to the well "G" and another two drops to the well "W".
6. **Gently** shake the test tray to mix the reagents within each well. Avoid spillover that will mix the reagents from the two wells.
7. Put the test tray with the reagents in a microwave oven.
(Tips: the required heating time is shorter if the test tray is set at the edges of the rotating tray, not at the center).
8. Set heating time for 30 seconds. Start heating.
9. Monitor the heating process, once you see a color change or any liquid splatters with the sample "G", you may stop the heating. (The required heating time depends on the power of the microwave oven, usually it takes no more than 30 seconds).
10. Record color changes in Table 2.
11. To avoid staining, rinse the tray with tap water and wipe the tray with a paper towel. Keep the tray for the next test.

Station 3 - Carbohydrate Testing: Starch

A. Material

- 6-well white test tray
- Bottle of Iodine Solution
- Bottle of DI Water (Control)
- Bottle of Starch Solution
- Paper towel for cleaning
- Fine point permanent marker

B. Procedure

1. Set the 6-well white test tray on the table.
2. Label a clean well from the test tray "S". Label a second clean well "W". See picture on assay station cards provided.
3. Drop two drops (~25 μL per drop) of the **Starch Solution** to the well "S".
4. Drop two drops (~25 μL per drop) of the **DI Water** to the well "W".
5. Drop two drops (~25 μL per drop) of the **Iodine Solution** to the well "S" and another two drops to the well "W".
6. Record color changes in Table 2.

Station 4 - Protein Testing

A. Material

- 6-well white test tray
- Bottle of Biuret Reagent
- Bottle of DI Water (Control)
- Bottle of Protein Solution
- Paper towel for cleaning
- Fine point permanent marker

B. Procedure

1. Set the 6-well white test tray on the table.
2. Label a clean well from the test tray "P". Label a second clean well "W". See picture on assay station cards provided.
3. Drop two drops (~25 μ L per drop) of the **Protein Solution** to the well "P".
4. Drop two drops (~25 μ L per drop) of the **DI Water** to the well "W".
5. Drop four drops (~25 μ L per drop) of the **Biuret Reagent** to the well "P" and another four drops to the well "W".
6. **Gently** shake the test tray to mix the reagents within each well.
7. Wait for about 1 minute to allow any color change to fully develop.
8. Record color changes in Table 2.

Station 5: DNA Testing

A. Material

- 0.65 mL clear microcentrifuge tubes (2 tubes per student group X number of groups)
- Set one tube of "GelGreen DNA Stain", one tube of "DNA solution" & one tube of "DI Water" on a microcentrifuge tube rack
- Micropipette (20-200 μ L adjustable volume)
- Rack of micropipette tips, or provide at least 30 pipettes tips
- Fine point permanent marker
- Paper cup or beaker to hold used pipette tips
- The Winston Fluorescence Reader with orange photo hood (one to two sets)
- Optional - centrifuge

B. Procedure

1. Use two clear microcentrifuge tubes for this test. Label one tube "DNA" and the other tube "W". See picture on assay station cards provided.
2. Set a micropipette to 20 μ L. Apply a clean pipette tip to the micropipette.
3. Transfer 20 μ L of **GelGreen DNA stain** to **each of the two** microcentrifuge tubes. Discard the pipette tip.
4. Apply a clean pipette tip to the micropipette. Transfer 20 μ L of **DI water** to the tube "W". Discard the pipette tip.

5. Apply a clean pipette tip to the micropipette. Transfer 20 μL of **DNA solution** to the tube "DNA". Discard the pipette tip.
6. Cap both tubes tightly, gently flick the tubes to mix, then centrifuge briefly to bring all the liquid to the bottom of the tubes. If you do not have a centrifuge then tap the tube on a benchtop to bring as much of the reagent to the bottom of the tube.
7. Place the two tubes in **The Winston Fluorescence Reader** (or a blue light illuminator). Set the orange photo hood on the reader. The blue LED lights inside the reader will turn on automatically.
8. Observe the color of the liquid, record results in Table 2 and dispose of tubes.

Notes: Now go back to the Lipid Testing (at Station 1), step 7 to compare and record the results in Table 2.

Table 2: Test Observation & Result

Macromolecule	Test Reagent / Test	Positive Result	Control (Negative) Result
Lipid - Oil	Paper Spot		
Carbohydrate - Glucose	Benedict's Solution		
Carbohydrate - Starch	Iodine Solution		
Protein	Biuret Reagent		
DNA	GelGreen DNA Stain		

Name: _____

Date: _____

Worksheet 3 : Exploratory Activities

You have learned how to test for the different biological macromolecules in the previous section, it is time to apply your knowledge to help solving the following questions:

(Each students group may pick one of these questions or work on all of them)

- 1. Three unlabeled tubes:** Dr. Smith is working in her preparation room when she notices a leak in the ceiling. Water has dripped onto the labels of three different sample tubes, causing the labels to come off. The affected tubes are now unlabeled and are referred to as unlabeled tube #1, unlabeled tube #2, and unlabeled tube #3. Dr. Smith is confident that each tube contained one of the following substances: sucrose, glucose, or starch. Can you help Dr. Smith identify the contents of each sample tube and re-label them correctly? Think – under what category do these samples fall into? Test the supernatant for the macromolecule(s) you think should be tested.
- 2. Urine Trouble!** Uncle Sam who is 67-year-old and has retired since last year. He does not know why he always feels thirsty and extremely hungry recently. Even though he eats a lot more than before, he has lost over 30 pounds. He wonders what has happened to his body? Can the students help him on this matter? A urine test may give him the answer.
- 3. A tube contains "Vomit Substance": Is Kyle guilty or innocent?**
Kyle's mom, Sarah, has prepared dozens of chocolate chip cookies for Kyle's soccer game. Shortly before leaving for the game, Sarah finds that someone has eaten a large portion of the cookies, and next to the cookies is a pile of vomit on the floor. Kyle admits to vomiting due to feeling sick, but insists he vomited due to his dinner, which consisted of steak and potatoes, not the cookies. He claims he has no idea how the cookies disappeared. We have obtained a sample of his vomit; can we find out if Kyle is telling the truth?
Test the supernatant for the macromolecule(s) you think should be tested.
- 4. What is included in your cat's or dog's food? Does your pet get enough protein from the food? Does this pet food have a lot of grains which are not good for the health of your cats & dogs?**
Let's find out! First resuspend the dry pet food powder in 5 mL DI water, transfer 65 uL into a 0.65 mL microcentrifuge tube, balance then centrifuge the tube to separate the liquid from the solids. The liquid, or supernatant, should be tested for the macromolecules you think should be tested.
- 5. There are so many different protein powders in the market, which one gives you the best value and most protein per serving?** Students will analyze two brands of protein powder and make recommendations based on their findings. First add 5 mL DI water to dissolve P1 and then add 5 mL DI water to dissolve P2 separately. Transfer 65 uL of each into separate 0.65 mL microcentrifuge tubes, balance then centrifuge the tubes to separate the liquid from the solids. The liquid, or supernatant, should be tested for the macromolecules you think should be tested.
Question: Which protein powder gives the better value to the consumer?

Worksheet 3 : Exploratory Activities

Question # _____: _____

1. Test Plan

2. Test Procedure


3. Test Results

4. Conclusions

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