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MINILABS

Taking Macromolecules to Micro MiniLab

Student Worksheets

Cat# M3014

Version 082321

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		

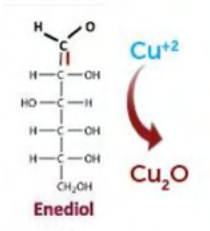
Test Reagent Guide

Benedict's Solution:

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 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:

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:

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 petite 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:

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 macromolecules. Each students group will take turns to go around each station to perform the tests and record the result in data table 2.

Station 1 - Lipid Testing: Oil

A. Material

- Set one tube of "Oil" & one tube of "DI Water" on a microcentrifuge tube rack
- Oil Test cards (1 piece per student group X number of groups)
- One micropipette (2-20 or 20-200 µL adjustable volume)
- Rack of micropipette tips, or provide at least 20 pipettes tips
- Paper towel for cleaning
- Fine point permanent marker
- Paper cup or beaker to hold used pipette tips

B. Procedure

1. Take an Oil Test card and label with your group name.
2. Set a micropipette to 20 µL. Apply a clean pipette tip to the micropipette.
3. Pipette 20 µL of water on to the center of the circle labeled as "Water". This is the negative experimental control.
4. Discard the used pipette tip into the beaker/paper cup.
5. Apply a new pipette tip to the micropipette, transfer 20 µL of oil on to the center of the circle labeled as "Oil". Discard the used pipette tip.
6. Allow the drops to sit and air dry (takes at least 10 minutes).
7. 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".
3. Add two drops (~25 µL per drop) of the **Glucose Solution** to the well "G".
4. Add two drops (~25 µL per drop) of the **DI Water** to the well "W".
5. Add 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 spill over that will mix the reagent 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 with the sample "G" or the liquid sputter or even a sparkle, 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 paper towel. Keep the tray for 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

A. 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".
3. Add two drops (~25 µL per drop) of the **Starch Solution** to the well "S".
4. Add two drops (~25 µL per drop) of the **DI Water** to the well "W".
5. Add 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".
3. Add two drops (~25 µL per drop) of the **Protein Solution** to the well "P".
4. Add two drops (~25 µL per drop) of the **DI Water** to the well "W".
5. Add four drops (~25 µL 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 µL 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)

B. Procedure

1. Use two clear microcentrifuge tubes for this test. Label one tube "DNA" and the other tube "W".
2. Set a micropipette to 20 µL. Apply a clean pipette tip to the micropipette.
3. Transfer 20 µL 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 µL 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.
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		

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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. Sucrose is a disaccharide, a molecule composed of two monosaccharides: glucose and fructose. Can you use one or two of the tests to identify the presence of sucrose in the provided solution? If not, why?

2. Find out what type of macromolecule(s) is/are in the provided tube which contains the vomit substance. Your group will first need to resuspend the "vomit substance" in 5 mL DI water and then test the supernatant for various types of macromolecules.

3. 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 your group help him on this matter? A urine test may give him the answer.

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.

   A tube of pet food powder is provided and your group will first need to resuspend the dry powder in 5 mL DI water and then test the supernatant for various types of macromolecules.

5. There are so many different protein powders in the market, which one gives you the best value and most protein per serving? Your group will analyze two brands of protein powder and make recommendations based on your findings. Students need to set up a test plan to compare protein amount in solution. You will add 5 mL DI water to dissolve P1 and then add 5 mL DI water to dissolve P2 separately. The supernatant in each tube can be used to test for the presence of protein and other types of macromolecules.

Worksheet 3 : Exploratory Activities

Question #_____: _____

1. Test Plan

2. Test Procedure

3. Test Results

4. Conclusions

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