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A Geno Technology, Inc. (USA) brand name

Dialysis

Teacher's Guidebook

(Cat. # BE-603)

MATERIALS INCLUDED

This kit has enough materials and reagents for 30 students (six groups of five students.)

- 1 bottle Starch Solution
- 1 bottle Glucose
- 1 bottle Starch Indicator Solution (Iodine Solution)
- 1 bottle Benedict's Solution
- 10 Centrifuge Tubes (1.5ml)
- 6 Tube-O-DIALYZER™ Devices (Micro)
- 6 Tube-O-DIALYZER™ Holders
- 6 Dialysis Cups
- 18 Glass Balls

SPECIAL HANDLING INSTRUCTIONS

- All reagents can be stored at room temperature

ADDITIONAL EQUIPMENT

- Waterbath or beaker and thermometer

TIME REQUIRED

- **Day 1:** 2 hours
- **Day 2:** 1 hour

AIMS

- Understand the principles of dialysis.
- Monitor the bidirectional flow of molecules through a semi-permeable membrane.
- Use colorimetric tests to study the movement of molecules.

BACKGROUND

Dialysis is the diffusion of a solute across a selectively permeable membrane and is routinely used in research and medicine. In research, dialysis is used to separate molecules in solution and the most common use in medicine is in renal (kidney) replacement therapy.

In research, a solution of several types of molecules are placed into a semi-permeable dialysis bag, such as a cellulose membrane that has pores (small holes). The sealed dialysis bag is placed in a container of a different solution and molecules small enough to pass through the tubing move into or out of the dialysis bag, in the direction of decreasing concentration. Larger molecules, including proteins, DNA, or polysaccharides that have molecular weights significantly greater than the pore diameter are retained inside the dialysis bag. One common reason for using this technique would be to remove the salt from a protein solution. Dialysis membranes are rated by the size of molecules that they retain, this is known as the Molecular Weight Cut Off (MWCO)

The principle is the same in renal replacement therapy, where a semi permeable membrane separates the blood flow from a dialysis solution. The body's waste products pass through the membrane and are removed from the blood.

The following experiment allows students to understand the bidirectional movement of molecules from an area of high concentration to an area of lower concentrations and also to understand the retention of molecules that have molecular weights greater than the MWCO.

Students will set up dialysis experiments using starch, iodine and glucose and monitor their diffusion using colorimetric assays.

MATERIALS FOR EACH GROUP

- Starch Solution
- Starch Indicator Solution (Iodine Solution)
- Glucose Solution
- Benedict's Solution
- 9 Centrifuge Tubes (1.5ml)
- 1 1000 MWCO Tube-O-DIALYZER™ Device
- 1 Dialysis Cup
- 1 Tube-O-DIALYZER™ Holder
- 3 Glass Balls

PROCEDURE

Glucose and Starch Assays

The aim is to test the color indicator assays, prior to the dialysis experiment, in order to aid in interpretation of the results.

1. Label seven 1.5ml centrifuge tubes with your group's name and number them 1-7.
2. Aliquot in 0.5ml of the appropriate test solution as indicated in the table below. Ensure a clean pipette tip is used for each solution to prevent cross-contamination.

Tube #	Test Solution
1	Water
2	Starch Solution
3	Glucose Solution
4	Water
5	Starch Solution
6	Glucose Solution
7	Starch Indicator Solution (Iodine Solution)

3. Add 0.1ml orange Starch Indicator Solution (Iodine Solution) to tubes 1-3 and note the color changes in the results section.
4. Add 0.1ml blue Benedict's solution to tubes 4-7. Heat in a hot waterbath for 5 minutes. Carefully remove the tubes from the waterbath and note the color changes in the results section.

If necessary, this is a convenient stopping point.

Dialysis Experiment

The aim is to test dialyze a mixed solution of glucose and starch against a dialysis buffer and determine the movement of the molecules.

The experiment is conducted with Tube-O-DIALYZER™, a micro dialysis device. Tube-O-DIALYZER™ devices replace conventional dialysis bags as they are easier to load, use and recover samples. The conventional 1000MWCO dialysis membrane is located in the cap. Handle the devices carefully ensuring the dialysis membrane is not pierced or dislodged. Do not let the membrane dry out.

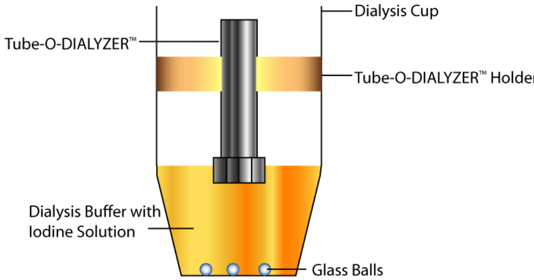

Figure 1: Dialysis Set Up

1. Label a Dialysis Cup with your group name. Place 3 glass balls into the Dialysis Cup to aid stirring.
2. Add 9ml distilled water and 1ml Starch Indicator Solution (Iodine Solution) to the Dialysis Cup.
3. Using a pipette, remove any liquid from the Tube-O-DIALYZER™ and discard.
4. Add 0.2ml water to the Tube-O-DIALYZER™ tube, add the cap and briefly shake to rinse the membrane. Remove all the liquid and discard.
5. Add 0.1ml Starch Solution and 0.1ml Glucose Solution to the Tube-O-DIALYZER™ tube and seal with the Tube-O-DIALYZER™ cap.
6. Place the Tube-O-DIALYZER™ in the Tube-O-DIALYZER™ Holder and invert and position in the Dialysis Cup as shown in figure 1.
7. Place the entire set up on a gentle shaker and shake overnight at room temperature.

If necessary, after overnight dialysis the samples can be stored in a fridge for up to 1 week. It is recommended that the tube contents are transferred to a fresh tube with a tight fitting lid and the cap is placed on the dialysis cup before storage.

Analysis of Dialyzed Samples

The aim is to use the Benedict's assay to identify the movement of the glucose molecules. The movement of the starch can be assessed by examining the colors of the dialysis solutions.

1. Note the color of the solution in the tube and the dialysis cup and record your observations in the result section.
2. Label a 1.5ml Centrifuge Tubes with your group name and "Dialysis Buffer". Transfer 0.5ml Dialysis Buffer from the Dialysis Cup to the tube.
3. Label a 1.5ml Centrifuge Tubes with your group name and "Tube Solution". Transfer 0.1ml solution from the dialysis tube to the tube.
4. Add 100μl Benedict's Solution to both tubes and place in a hot waterbath for 10 minutes. Note the color and record.

RESULTS, ANALYSIS & ASSESSMENT

Iodine Assay

Tube #	Test Solution with Iodine	Result
1	Water	*Pale orange*
2	Starch Solution	*Deep blue black*
3	Glucose Solution	*Pale orange*

Benedict's Assay

Tube #	Test Solution with Benedict's Solution	Result
4	Water	*Pale blue*
5	Starch Solution	*Pale blue*
6	Glucose Solution	*Cloudy rust color*
7	Starch Indicator Solution (Iodine Solution)	*Pale blue*

Discuss your results:

Iodine interacts with starch to produce a color change from orange to a dark blue/black color. The iodine does not interact with glucose or water, which makes it an ideal solution to test for the presence of starch.

Benedict's solution reacts with glucose upon heating to form a rusty brown precipitate. Benedict's solution does not react with starch, water or iodine, which makes it an ideal solution for testing for the presence of glucose.

Analysis of Dialyzed Samples

Describe the color of the dialyzed sample in the tube and discuss:

The solution in the tube is a deep blue black color, which is indicative of iodine interacting with starch. This shows that the small iodine molecules passed through the membrane into the tubes. In addition, the dialysis buffer is a very pale orange, indicating the presence of some iodine. This shows that the iodine can pass into the dialysis tube, but the starch cannot pass out. If starch had moved out the iodine would have reacted with the starch to turn blue black. Starch therefore has a molecular weight greater than 1000 daltons.

Benedict's Solution Assay

Tube #	**Result**
Dialysis Buffer	*Pale blue with an orange precipitate*
Tube Solution	*Dark Rusty Orange Color*

Discuss your results:

The formation of a red brown precipitate in the dialysis buffer shows that glucose has migrated through the membrane. The low amount of precipitate is due to the dilution of the glucose. Due to the glucose being able to migrate back and forth through the membrane and equilibrium is obtained as a result the glucose is found on both sides of the membrane. Glucose passes through as it is a smaller molecule than starch.

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Dialysis

Student's Handbook

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PROCEDURE

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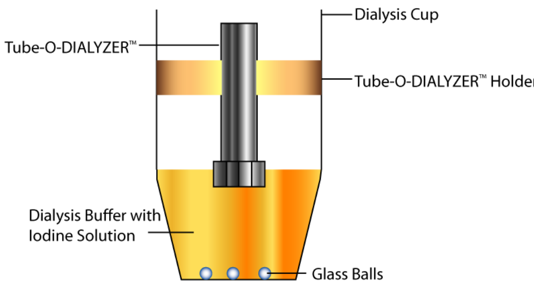

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4. Add 0.2ml water to the Tube-O-DIALYZER™ tube, add the cap and briefly shake to rinse the membrane. Remove all the liquid and discard.
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6. Place the Tube-O-DIALYZER™ in the Tube-O-DIALYZER™ Holder and invert and position in the Dialysis Cup as shown in figure 1.
7. Place the entire set up on a gentle shaker and shake overnight at room temperature.

If necessary, after overnight dialysis the samples can be stored in a fridge for up to 1 week. It is recommended that the tube contents are transferred to a fresh tube with a tight fitting lid and the cap is placed on the dialysis cup before storage.

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3. Label a 1.5ml Centrifuge Tubes with your group name and "Tube Solution". Transfer 0.1ml solution from the dialysis tube to the tube.
4. Add 100µl Benedict's Solution to both tubes and place in a hot waterbath for 10 minutes. Note the color and record.

RESULTS, ANALYSIS & ASSESSMENT

Iodine Assay

Tube #	Test Solution with Iodine	Result
1	Water	
2	Starch Solution	
3	Glucose Solution	

Benedict's Assay

Tube #	Test Solution with Benedict's Solution	Result
4	Water	
5	Starch Solution	
6	Glucose Solution	
7	Starch Indicator Solution (Iodine Solution)	

Discuss your results:

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Analysis of Dialyzed Samples

Describe the color of the dialyzed sample in the tube and discuss:

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Benedict's Solution Assay

Tube #	**Result**
Dialysis Buffer	
Tube Solution	

Discuss your results:

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