

Testing the Aerobic Growth of *Halobacterium* in Light and Dark Conditions: A Student Laboratory Investigation

Questions: What environmental conditions cause *Halobacterium* to turn on or off the genes coding for the protein, Bacteriorhodopsin? Can the environment change an organism's phenotype?

Materials per group:		Classroom Materials:
3 50mL flasks	OR 3 50mL flasks covered in electrical tape	Magnetic stir station
1 tube containing 12mL of <i>Halo</i>	8 beral pipets (4 for day 1, 4 for day 2)	Lamp with a special full spectrum bulb
	3 microcentrifuge tubes	Kim wipes
20-50 mL graduated cylinder	Permanent Marker	cardboard to make stabilizer
1 bottle with 60 mL of CM	Labeling tape	Microcentrifuge
3 cuvettes	foil for caps (and covering)	Distilled water for clean up
3 stirring bars	electrical tape if covering flasks	Spectrophotometer

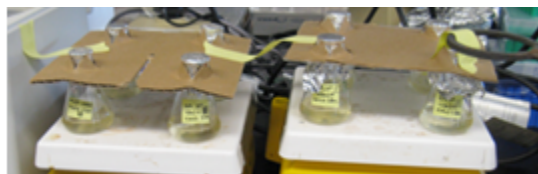
VERY IMPORTANT Before using any non-sterile labware, rinse thoroughly with hot tap water and then a final rinse with deionized water. *Halo* do not tolerate soap!

Procedure (Day 1):

1. Clean your graduated cylinder and measure 20 mL Complete Media (CM). Do not over-pour. Under-pour and use one beral pipet to add the appropriate amount of CM.
2. Pour the CM into a flask. Repeat so that you have 3 flasks (4 if your teacher asks you to make a control flask).
3. Gently swirl the *Halobacterium* culture (in the tube). Use a beral pipet to add 4 mL *Halo* culture to each of your flasks.
4. Make a foil cap for your flask and gently swirl the flask to ensure the sample is evenly mixed.
5. Repeat steps 3 and 4 for your other sample flasks.
6. Using the labeling tape, label your flasks with your group name, the organism's name (*Halo*), the date, the sample number, and the condition being tested (either light or dark). Keep in mind that light will shine through your culture, so do not block it with your label.
7. Take the beginning optical density (OD) of your culture. To do this, remove the foil cap and then:
 - a. Use a new beral pipet for each of your samples to place the correct amount into a separate clean cuvette.
 - b. Set the spectrophotometer to absorbance and to a wavelength of 600 nm.
 - c. Use Kim wipes when handling cuvettes.
 - d. To zero the spectrophotometer, insert provided blank, press blank button and **wait** for zero to appear.
 - e. Insert cuvette with sample; observe absorbance reading. Record these numbers as the beginning optical density. If your numbers are not nearly identical for all 3 samples, describe possible reasons why in your lab notebook.
 - f. Pour your sample back into the appropriate flask for incubation.

g. Repeat with each of your samples.

8. Place a clean stir bar in each flask and put the foil caps back on and place your samples on stir stations according to your teacher instructions. (Stir setting should be on the lowest possible and a device (cardboard form) to hold the flasks in place is needed).



9. In your lab notebook, note the date and time you started the incubation. Make a prediction about what will happen to the cell growth and resulting absorbance in the future days. Also, predict how the sample may change in appearance.

Procedure (Day 2): You will be taking an absorbance reading and spinning down cells.

1. After at least 72 hours, obtain your three samples. Record the time and date the incubation stopped.

To measure your cell density:

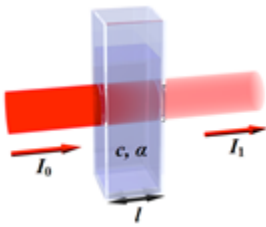
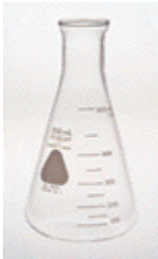
2. Use a clean beral pipet to place a sample from one of the flasks into a clean, empty cuvette. (Use the same beral pipet to place a sample, from the same flask, into the microcentrifuge tube.) Repeat for each flask but use a new beral pipet each time.
- See the procedure in step 7 from Day 1 for measuring the absorbance of each sample. **Run the blank before each sample.**
 - Record data in your data table.

To spin down your cells:

- Using the appropriate beral pipet, transfer 1.5 mL from your sample to an empty microcentrifuge tube. You can use the markings on the pipet to first draw out 1 mL, then 0.5 mL.
 - Snap the lid on the microcentrifuge tightly closed.
 - Use a permanent marker to label the outside of your microcentrifuge tube with your group name, the sample number and the sample type (light or dark).
 - Repeat this for each of your samples.
 - Take your samples to the microcentrifuge. Symmetrically place tubes inside a centrifuge so they balance each other out, otherwise the centrifuge may break.
 - Close the lid and turn the centrifuge on for 15 minutes at 6,000 rpms. (This is a good time to make sure your lab book is current, or to take absorbance readings.)
 - Remove your tubes from the microcentrifuge and locate the cells (pellet) at the bottom of the tube. Without agitating the cells, use a beral pipet to remove and discard the liquid above the cells.
3. **RETURN CUVETTE SAMPLE TO THE APPROPRIATE FLASK - DO NOT DISCARD.** Ask your teacher where to keep your flasks and your cell pellets. You will need the cell pellets for your next lab activity. Flasks should be kept until you are sure you have no further need for them.

4. Clean up your lab space and equipment.
 - a. Do NOT USE SOAP when cleaning equipment.
 - b. Rinse cuvettes thoroughly three times with hot tap water.
 - c. Rinse one more time with deionized water.
 - d. Return equipment to proper locations designated by the teacher.

Materials Identification:

 Plastic cuvette (typically needs 1mL)	 Glass cuvette (typically needs 3 mL)	 Spectrophotometer	 Graduated cylinder	 Beral pipet
 Microcentrifuge tube (epi tube)	 Microcentrifuge	 Flask	 Magnetic stir plate	 Magnetic stir bar