Please note: The* Halobacterium *culture <u>should have been</u> prepared AT LEAST 4 days prior to beginning the lab with students. The directions for this are located below in the section entitled, "Preparation of the Culture."

Safety Equipment	Materials per Group	Equipment per Group	Class Equipment
Gloves	Deionized water	Spectrophotometer	Shaker Incubator
Goggles	Complete Medium (CM)	4 cuvettes	
Apron	Live *Halobacterium* culture	Large rack for cuvettes/Falcon tubes	
	Falcon Tube Labels	Kim wipes	
		Beral pipet	
		P200 micropipet w/tips	
		400-1000 mL beakers for waste	
		4 Falcon Tubes, 17x100 mm	
		Sharpie marker	

<u>TEACHER PREPARATION INSTRUCTIONS FOR THE *HALOBACTERIUM* SALINITY LAB</u>

PREPARATION OF THE CULTURE – Note: Please read through the entire direction set before preparing your culture. Important and needed instructions are clarified and more specifically stated below the item with bulleted points.

1. You must first prepare the *Halobacterium* culture for the students to use. This is a fairly easy process, but care must be taken in order to prepare a viable sample for the students to use.

2. Open the kit **at least 4 days** prior to when the students are completing the lab.

3. Use one of the enclosed, sterile disposable pipets to add 5 mL of 4.3 M growth medium (from the stock solution bottles in the kit) to 1 Falcon tube. Repeat this step again with a second Falcon tube. Since the graduations on the pipet only go to 1 mL, you will have to transfer 1 mL, 5 times. Be sure to avoid contamination. Using this 1 pipet and the 10 mL of 4.3 M will allow your students to have enough media to complete their experiment. However, if you have a glass pipet and bulb or a suitable micropipette, feel free to use that if you prefer. Make sure there is no soap residue in anything you use. Soap will cause the *Halobacterium* to lyse.
    - Do not add more than 5 mL of growth medium to the Falcon tube. The cells need to have a great deal of empty "air" space in the tube so that oxygen can be forced to cycle through this high saline sample without losing water through evaporation.

4. Locate the agar stab enclosed in the kit. This is contained in a Falcon tube and is labeled, "*Halobacterium* Culture." You should see pink colonies growing on the surface of the agar.

5. Use the enclosed toothpick to GENTLY remove a single pink colony from the agar. Carefully remove the toothpick (containing the colony) from the Falcon tube and gently **drop the entire toothpick and colony into** the Falcon tube that has the 5 mL of growth medium in it. The toothpick should remain in the Falcon tube while the culture is incubating. Place the Falcon tube cap TIGHTLY on the tube (to the second stop) by firmly pressing the cap with your thumb. When completing this, **pay careful attention** to the bulleted points below that further describe this step.

- If red colonies are present, do not use them. The pink colonies have intact gas vesicles and will form a more viable culture for your students to use.
- It is best to only remove 1 colony with your toothpick. This is because it is likely these organisms are identical genetically. If only one colony is used, we will be limiting the variables in this experiment. However, it is okay if you remove more than 1 colony.
- Try not to transfer agar with the colony. A gentle swabbing motion with the toothpick (instead of a gouging motion) should achieve this. If some agar is transferred, your culture will likely still be viable.
- You should have 2 toothpicks in your kit. However, if toothpicks are not available, you may use a similar object (such as a micropipette tip). Be certain that the object is small enough to allow the Falcon tube to tightly close. We are not terribly worried about contamination in this step, because it is unlikely that anything besides *Halobacterium* will be able to grow in the 4.3 M medium.
- There are 2 "stops" to closing a Falcon tube. Be certain that the cap is closed to the 2nd stop (as tightly as it can be). Due to the high saline solution, evaporation is likely. This cap placement prevents a great deal of evaporation while the shaking motion of the incubator encourages enough oxygen to cycle through the sample.

6. Using a new toothpick and your second Falcon tube containing 5 mL of growth media, repeat the above steps listed in step #5.

7. Take the two inoculated Falcon tubes and place them in a shaking incubator at 37°C and 220 rpm for 3-4 days.

8. Remove the Falcon tubes from the incubator. You now need to test the sample to make sure you have enough cells for your experiment.
    - Set the mode of your spectrophotometer to ***absorbance*** and to a wavelength of ***600 nm***. This will allow you to measure the optical density (OD) of your sample.
    - Transfer a sample of your inoculum to a clean cuvette. Use the guidelines listed below.
        - Be certain to use a sample size that is appropriate for your spectrophotometer. For the "Fisher Visible Spectrophotometer," at least 2.5-3.0 mL must be used.
        - In order to clean your cuvette (before and after testing the OD), use copious amounts of deionized water. Do not use soap on the cuvette. If there is soap residue on the cuvette, it will cause the cells to lyse.
        - It is okay to simply pour your sample into your cuvette if it holds 5 mL.
        - Use Kim wipes to remove any fingerprints, etc. from the exterior of the cuvette.
    - Use a sample of your 4.3 M growth medium to blank your spectrophotometer. Again, use an appropriate amount for your spectrophotometer.
    - Insert your sample. Record your optical density. Repeat with your second culture.
        - Ideally, the OD should read between **0.6 and 0.8** for a great sample. If your OD is lower than 0.6, place your samples back in the incubator for another 24 hours and run the OD again. The sample can be used if the OD is as high as 1.2 or 1.5, but this is not ideal.
9. Return your samples to the Falcon tubes.
    - Leave your culture at room temperature. Be certain the cap is tightly on the Falcon tube and keep the sample out of direct sunlight. The cells will continue to grow very slowly. If your sample's OD is extremely high, you may choose to dilute it. However, remember to use 4.3 M medium, not water.
    - Before using the culture in the lab, gently swirl the solution to thoroughly mix the cells.
    - The original stab can remain at room temperature and will remain a viable culture source for many months.