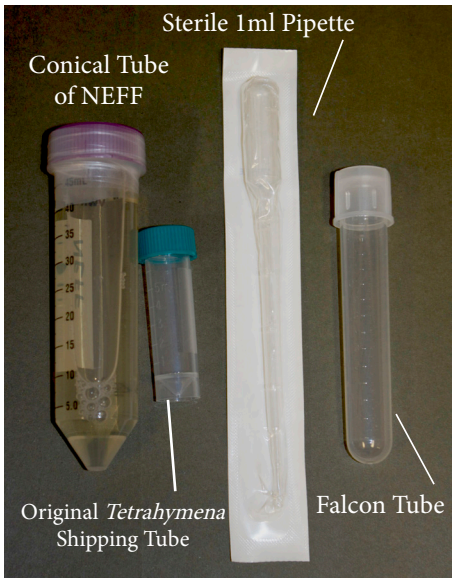
ASSET: How to Make Stock Cultures

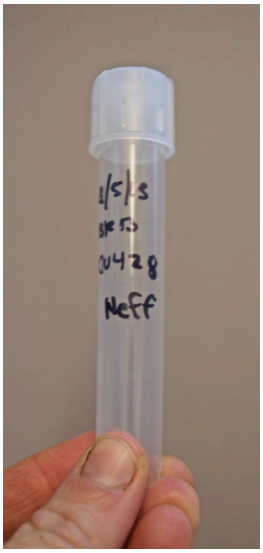
1. We recommend checking your cells upon arrival, use a sterile pipette to place a small drop of culture from your *Tetrahymena* shipping tube onto a microscope slide. Look for actively swimming *Tetrahymena* on the lowest objective.



2. Starting and maintaining clean stock cultures of your *Tetrahymena* will ensure a ready, usable supply of cultures for all your ASSET lab module needs. Your stock culture is your "back-up" culture in case of contamination.

3. Shown to the right are the materials we provide to start your stock culture. You should inoculate your stock culture tubes within 24-48 hours of receiving your kit. After 24 hours of incubation at room temperature, the stock culture can be used to make your classroom working cultures. Stock cultures should be kept until you are completely finished with your ASSET lab(s). They last 2-4 weeks at room temperature.

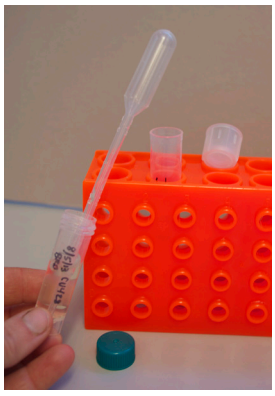
4. To begin making your stock culture, **wipe down your prep bench or counter with alcohol or a weak bleach solution and wash your hands before handling the cultures**. Make sure you have all your materials ready to make the culture and use only the sterile materials provided to minimize contamination.



5. Label each sterile Falcon tube with the following information: *Tetrahymena* strain from shipping tube, date of inoculation, media type (Neff or Tris), and your initials (see image to the left).

6. The Falcon tubes will arrive with the caps pushed tightly closed. When you are ready to use them, uncap the tubes and just lightly re-cap in anticipation of your culture transfer. Using one of the sterile pipettes, pipette 6 mL of sterile Neff media into each Falcon tube. Once you have pipetted the media into the tube, put the caps back on both the media and Falcon tubes.

7. Using a new sterile pipette, pipette 0.5 mL of *Tetrahymena* from the shipping tube into each of the Falcon tubes. Cap the Falcon tube but do not push it down all the way. You should be able to move the cap but it should not come off. This is to ensure gas exchange and aeration in your stock culture tube.



8. Store your stock culture tubes at room temperature in a spot that will not experience a lot of temperature fluctuations, i.e., not next to the window.

9. When you check your culture the next day, it will look a little cloudy. You may be able to see the *Tetrahymena* swimming around when you hold the tube up to the light. The *Tetrahymena* will keep in the stock tube for 2-4 weeks.

10. Accumulation of dead cells at the bottom is normal. When you are ready to inoculate your working cultures, use your remaining sterile pipette to transfer the *Tetrahymena* from the middle of the tube, do not shake to mix the tube or pipette from the bottom. You want to use live *Tetrahymena*.

11. Once you are done making your stock culture tubes, give the used pipettes and tubes a quick rinse with soapy water or a weak bleach solution before disposing.

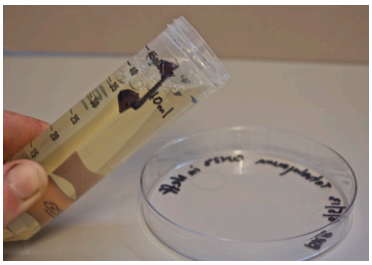
The ASSET Program (Advancing Secondary Science Education thru *Tetrahymena*) at Cornell University is funded by NIH SEPA

ASSET: How to Make Working Cultures

1. The working culture is the *Tetrahymena* culture your students will use in their lab. Working cultures should be made **3 days before your lab start date**, so work backwards from when you want to begin the experiment. **Working cultures last about 4-5 days at room temperature.** The picture to the right indicates the materials you received to make your working culture.

2. Using sterile petri plates, turn over the plates and label the bottom of each with the date, your initials and *Tetrahymena thermophila.*

3. We recommend using 2-4 petri plates per class of students. With smaller groups, 10-22 students, two petri plates should be sufficient; with larger groups you may require more plates or flasks of culture.

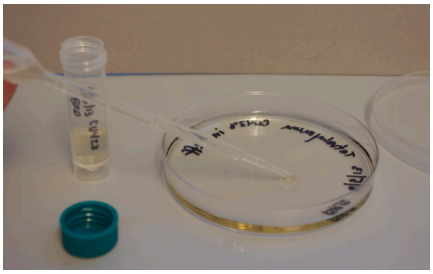


4. Measuring carefully, pour 10-15 mL of Neff into each petri plate. Try to do everything in one pouring so Neff doesn't become contaminated by running down the outside of the tube and then into the next plate. *(NOTE: Some labs require larger volumes of cells . Please consult the lab protocol for the module you are using. These instructions are for a standard ASSET Lab.)*

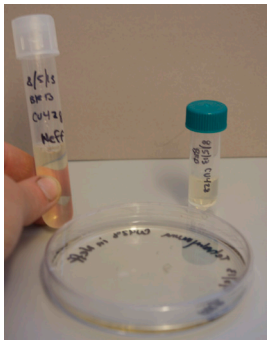
5. We send a minimum of 50 ml of Neff with each kit. After pouring your first working culture, you should have 35-40 ml of Neff remaining. Please be conservative with this liquid. Replace the cap on the Neff to minimize contamination. **DO NOT return opened Neff to ASSET!**

6. Using your stock tube, which has been incubating a minimum of 24 hours, and a sterile pipette, transfer 1-2 ml of your stock culture into each of your Neff-filled petri plates. Cover the petri plate and place it at room temperature in a location where it will not be disturbed. Let the culture grow for 2-3 days. It will appear slightly cloudy as the *Tetrahymena* increase in density.

7. Once you are done with a working culture, squirt a small amount of bleach or rubbing alcohol into the dish to kill the *Tetrahymena*, then you may clean the petri plate for a later, non-sterile use, or dispose of the plate in the trash.



8. **EXCEPTION TO THIS PROTOCOL:** If your materials arrive on a Thursday/Friday OR you don't set-up your stock culture until then, but need working cultures for Monday, use the **original *Tetrahymena* sample in your shipping box to make both your Stock and Working Cultures**. Just use a sterile pipette to divide the sample equally among the petri dishes and the stock tube. See the image to the left.



9. If you have multiple lab groups in a week, make sure that you have made working cultures for each group 3 days in advance. For instance, if you have a lab group on Tuesday, start working cultures the Friday before. If you have a second lab group on Thursday, start their working cultures on the Monday before they will be used.

10. To minimize contamination, always keep the lids on containers unless transferring liquid. If your cultures become contaminated, they will look abnormally cloudy. Sometimes there will be white floating debris or fuzzy, hairlike threads of fungus in the medium. Add bleach and dump them down the drain. **DO NOT send any liquid medium that has been opened back to ASSET.**

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