

Protocol for Preparing Solutions

1. Ensure your work area is clean and sterilize any surfaces that will be used with a generous amount of ethanol.
2. Gather supplies:
 - ☐ Gloves
 - ☐ Ethanol spray bottle
 - ☐ Paper towels or kimwipes
 - ☐ Permanent marker
 - ☐ Scooper
 - ☐ Stir bar
 - ☐ Vessel for mixing
 - ☐ Smaller storage vessels like falcon tubes
 - ☐ Stir plate
 - ☐ Microscale
 - ☐ Weigh boat/weigh paper
 - ☐ Sterile seawater or DI water
 - ☐ Graduated cylinder
 - ☐ Chemicals
3. Ensure all supplies are sterile. (*This is essential when preparing stock solutions. Contamination in the stock will likely result in contamination issues in tanks/cultures before you know it is in the stock, as well as result in wasted chemicals.*)
4. Use the chart below to plan out the volume of stock you will need to make based on the number of tanks/cultures you will be dosing.
5. Measure and pour the sterile seawater or DI water, as indicated in the **Media and Solutions** table, into the mixing vessel.
6. Sterilize the stir bar using ethanol and a wipe, let the ethanol evaporate, and add it to the mixing vessel.
7. Turn on the stir plate to a speed fast enough to generate a funnel in the water.
8. Turn the microscale on and add the weigh boat.
9. Zero the scale.
10. Using a scooper, carefully weigh out the chemicals needed based on the "Concentration of Stock" column on the **Media and Solutions** chart.
 - Weigh the chemicals in small increments because excess chemicals that have been removed from the original container must be thrown away, not returned to the container. Otherwise, you risk contaminating your whole bottle of chemicals.
11. Pour the weighed chemical into the mixing vessel a little bit at a time. (Avoid adding all the chemicals at once, as it may create clumps that will take longer to dissolve.)
12. Wipe up any spilled chemicals from your work area.
13. Allow the solution to stir until everything is dissolved.
 - You may need to reduce the stir speed to clearly see if everything is dissolved.
14. Pour the solution into smaller storage containers.
 - Use smaller containers to avoid opening the main stock container every time you use it. Opening the stock container frequently increases the chances of contamination.
 - GreenWave typically uses 50 mL Falcon tubes to store our solutions.
15. Label small containers with the date, chemical, concentration, and your initials.
16. Store the solution according to the **Media and Solutions** chart.
17. Dose cultures and tanks with the stock solution according to the respective "Concentration in Cultures" and "Concentration in Tanks" columns on the Media and Solutions chart.