

LAB PROTOCOL: HOW TO MAKE ALGAL BALLS

PREPARATION - BY LAB TECHNICIAN

- 1 To make a 3% solution of sodium alginate, add 3g sodium alginate to 100mL of distilled water in an Erlenmyer flask. Stir continuously for at least 4 hours, or overnight using a magnetic stirrer. Do not apply any heat.
- 2 To make 500mL of 2% calcium chloride, add 10g of calcium chloride to 500mL of distilled water and stir to dissolve.
- 3 You will need approximately 400mL of relatively dense chlorella culture to make 120mL of the chlorella-alginate mixture. Using this solution, you should be able to make at least 500 algal balls; enough for a class. To learn how to culture chlorella, visit our [Living Organism Care - Algae resource](#) on our website. Once your chlorella culture has grown enough to be a bright to darkish-green, siphon out as much of the denser areas into a measuring cylinder. Leave the culture to rest overnight to allow the algae to settle to the bottom of the cylinder. After this time, there should be a dark "plug" of dense algae at the bottom.



Figure 1: Supernatant separated from "plug" of dense algae

- 4 To prepare the chlorella-alginate solution, take note of the volume of the plug, then carefully remove the supernatant until it accounts for 2/3 of the total volume in the measuring cylinder, with 1/3 dense alginate. For example, if you have 10mL of chlorella down the bottom, remove all but 20mL of supernatant so that you are left with 30mL in total. Keep the supernatant aside. If the mixture is too thick later to drop easily through the syringe, then you can use this to thin it out a little. Mix the remaining chlorella and supernatant in the measuring cylinder and add to an equal volume of your sodium alginate, e.g., 60mL of chlorella and 60mL of alginate to make 120mL altogether.
- 5 To set up retort stand apparatus, attach a 30mL syringe barrel to the retort stand clamp so that the outlet is pointing downwards. Position the syringe barrel over a beaker of calcium chloride solution.
- 6 To make algal balls, transfer your algae/alginate mix to the syringe, and allow it to drop through into the solution. Keep at least 1.5mL of mixture in syringe so that gravity will help push it through. Check the rate of the drops and the shape of the balls as they fall to the bottom.
 - If the rate is slower than a drop or two per second, add a small amount of the supernatant.
 - If the balls flatten on entering the liquid, the syringe is too high and should be lowered.
 - If the mixture comes through the syringe is too low, you may end up with "sausages" of algae rather than balls.
- 7 Leave the algal balls in solution for 5-10 minutes then rinse with distilled water. Store the algal balls in distilled water. Loosen the lid on the container to allow airflow. The chlorella within the balls is alive and therefore needs light to stay active. Place the balls in a well-lit area until required. Do not place them in direct sunlight, or expose them to too much heat. We recommend using the algal balls as early as you can; otherwise, they are best used within 2 weeks.



MATERIALS

- 3g sodium alginate
- 10g calcium chloride
- 400mL of (bright to darkish-green) chlorella culture
- Distilled water
- Retort stand apparatus
- 30mL syringe barrel
- Plastic pipette
- Measuring cylinder
- Large beaker

SAFETY PRECAUTIONS

- Wear appropriate personal protective equipment (PPE).
- Know and follow all regulatory guidelines for the disposal of laboratory wastes.
- Avoid direct contact with any culture.
- Wash your hands thoroughly after