

Bioluminescent Algae Information Sheet

At CCAP we keep our bioluminescent algae under laboratory conditions in filtered nutrient enriched seawater (L1) and in lighting controlled constant temperature rooms, **but** they can be maintained quite easily at home or in a school laboratory.

There are a few things which you will need:

Sterile seawater, or an artificial seawater and L1 nutrients

Water taken close to shore can be affected by contaminants from the land and diluted by fresh water after a heavy rainfall, therefore we recommend making artificial sea water. Sea salts can be bought from pet shops/aquariums and some garden centres. (Do not just use table salt) Tap water can contain too much chlorine etc., so instead use bottled mineral water (we use Volvic), and add between 30 to 33 grams of sea salts per litre. Mix thoroughly, it can take some time for the salts to dissolve into the water. This will produce artificial water similar in salinity to the sea. Once you have made up the artificial seawater, boil it for about 5 minutes with a lid on, or heat in a microwave. You don't want to evaporate the water as this will increase the salt concentration. The important thing is to kill any microorganisms which may eat the algae cells. Killing all the bacteria and possible fungal spores is not completely necessary as there is already bacteria present in the culture. This sterilising step is recommended, but an unopened bottle of shop bought mineral water should be clean enough.

Once the water is cool, add filter sterilised L1 nutrients.

Clean Containers

If you are using the algae for experiments you will need glass Erlenmeyer flasks plugged with bungs made from sponge or cotton wool. This keeps out dust, bacteria and fungi whilst allowing a free distribution of gases. It is best to use several medium sized flasks rather than one large one, to reduce the risk of losing the lot by contamination. When you are ready to use the flasks make sure you rinse them well. Do not wash them with washing up liquid as this sticks to the sides of the flasks and affects the media. You can sterilise the empty flasks by putting them dry into an oven and allowing them to heat up for 20 mins. Once they are cool you then add the prepared media to them. The pH should be fine if you use mineral water to mix the sea salts and should not need to be adjusted.

Any unused media can be kept in the plastic mineral water bottle and kept in the fridge (remember to mark the bottle or someone may get a shock if they drink it!)

Light and temperature requirements

These algae need a light source, for which daylight may be adequate. The best position for the culture vessels is near a north facing window, but care must be taken to ensure that direct sunshine never falls on them. Try to keep them at around room temperature (15°C to 22°C) if they get too hot they will die.

Another option is to use fluorescent lighting. Set with a timer to be on for 12hrs and off 12hrs. Halogen lighting should not be used as they produce too much heat and the wrong wavelength of light.

These bioluminescent algae have a circadian rhythm which controls their bioluminescence timing. They will only produce bioluminescence during their night cycle. You can fool them by placing them in a cupboard with lighting set by a timer to come on during the night and off during the day. During their night cycle they must be kept in absolute darkness.

Culturing

Our Bioluminescent algae are a species of dinoflagellate called *Pyrocystis lunula*. When your algae arrive, remove the parafilm from around the top of the algae containers and loosen the lids slightly. They will be fine kept in their original containers at the correct lighting and temperature for a few days whilst you prepare their growth medium.

Add the supplied algae in 10% increments to the fresh growth media (e.g. 25mls into 250mls of media).

It may take a few weeks for them to grow dense, but it will only take a week for them to adjust to their new lighting regime.

Disposal

It is important to kill any algal cells before disposal: this could be by adding bleach or autoclaving, then flushing down the drain with plenty of water. Living algae could potentially cause problems if it gets into the sewerage system.

And finally

To see them glow, they will need to be shaken gently. If shaken too vigorously, they use up all the luciferase, which is the catalyst for the bioluminescence reaction, and will need to rest for a while to replenish this.