EMPCO

Some things, though they are not in their nature fire,
nor any species of fire, yet seem to produce light.
Aristotle, 4th Century BCE

CARE RECOMMENDATIONS FOR BIOLUMINESCENT DINOFLAGELLATES

Bioluminescent dinoflagellates are a type of planktonic alga (plural: algae) inhabiting coastal ocean waters. Individual cells of *Pyrocystis* species are relatively large, and slightly discernible to the unaided eye. At night, bioluminescent dinoflagellates emit blue light, called bioluminescence, in response to movement in the water. Bioluminescence becomes visible only after nightfall, driven by a circadian rhythm which is entrained by light-dark cycles. Bioluminescence can only be observed during the entrained night period, and is best observed several hours after nightfall. Abruptly moving a culture of *Pyrocystis* from light to dark will not result in bioluminescence, due to the circadian control of this chemical reaction.

Arrival Instructions
Upon arrival, remove the bottle of algae from any plastic wrapping. Prior to manipulating the cultures in any way, or diluting with new growth medium, attempt for several nights to observe bioluminescence. Once bioluminescence has been observed, you may manipulate or dilute cultures as necessary. Please be aware that due to shipping stresses, bioluminescence may not be visible the first night after receiving the shipment.

For cultures packaged in **square bottles**, it is recommended to transfer the contents to a culture flask, such as an Erlenmeyer, for optimal growth. If a culture flask is unavailable, ensure the cap of the square bottle is tightened and orient the bottle horizontally on its side, rather than upright, to permit cells to settle by gravity over a large surface area. For cultures packaged in **Erlenmeyer flasks**, gas exchange is possible via the inset membrane. Erlenmeyer flasks should be oriented upright with caps tightened.

Algae require a light source for photosynthesis. Light can be provided by a full-spectrum compact fluorescent bulb or LED source. The light source must provide full-spectrum white light. Do **not** use blue/red LED lights optimized for green plant growth, or lamps which generate excessive heat (i.e. incandescent or halogen types). Do **not** place the culture in areas having exposure to direct or bright sunlight, or temperature fluctuations (such as in the vicinity of a windowsill).

Room temperature is optimal (from 18 ºC to 24 ºC or 65 ºF to 75 ºF), and temperature fluctuation beyond this range should be avoided (i.e. close to a windowsill). Overheating is generally more hazardous to bioluminescent dinoflagellates than cold, and exposure to direct sunlight should be avoided at all times. Each flask of algae contains nutrients, and given proper light and temperature, dinoflagellates should survive for at least one month (customers sometimes find that cultures survive for many months).

Culture Maintenance
To extend the lifespan of a bioluminescent algal culture, dilute an actively growing culture with new growth medium. It is best to perform dilutions prior to any decline in health of the culture to prevent the accumulation of dead biomass (cell detritus). First, swirl the culture to evenly suspend cells. Dispose of 50 to 70 percent of the volume of the culture, and replace with new growth medium. Bioluminescence of the diluted culture will decrease proportionally with the volume of culture discarded. However, as the diluted culture grows and returns to density over several weeks, maximum bioluminescence will be restored. The remaining algae will continue to multiply for approximately one to two months in the new growth medium.

Alternatively, to prevent long-term accumulation of biofilms on the base of the culture flask, new growth medium and algal culture may be combined in a new flask; an approximate dilution ratio of 4:1 (4 parts growth medium to 1 part algal culture) is recommended. To maintain an optimal liquid-to-air surface area interface for gas exchange in Erlenmeyer flasks, do not fill flasks beyond their recommended working volume (liquid should occupy only the lower bulbous portion of the flask).

If using a fluorescent lamp, an ideal light:dark cycle should be established. The light period should last 12 to 14 hours, followed by a dark period for the remainder of the 24-hour cycle. Bioluminescence can only be observed during the dark period. For best results, view bioluminescence an hour or more after the start of the dark period. Light cycles should be automatically controlled by a 24-hour lamp timer.

Additional Information
Dinoflagellates are a division (phylum) of algae. They are unicellular: each alga is comprised of only a single cell. Though a common misnomer, dinoflagellates are not plants, but algae. Unlike plants, algae are a large group of unrelated microbes that posses no vascular tissues (i.e. phloem and xylem) and no organs (i.e. roots, stems, or leaves). Algae are phylogenetically ancient in comparison to land plants, and gave rise to the plant chloroplast, via endosymbiosis of a green alga.

Algal bioluminescence has been misunderstood throughout history by some of the world's most famous scientists, including Aristotle, Boyle, Hooke, and Newton. Today, the purpose of bioluminescence in algae remains a mystery. Case et al. (1995) found evidence for the “burglar alarm theory”, which was proposed by Burkenroad in 1943: light emission reduces grazing by nocturnal predators by attracting predators of the grazers. Case determined that the feeding rate of squid on mysid (mysid are a predator of bioluminescent dinoflagellates) increased dramatically in the dark in the presence of bioluminescent dinoflagellates. In the absence of bioluminescence, squid were unable to effectively locate the mysid.