

## ***Nassula variabilis***

**Note:** The culturing conditions below are not necessarily the optimal growth conditions for each strain, as much variation is found between strains, and cultures are not always kept in optimal growth conditions at CCAP for practical reasons. There may be more info in the individual strain data on the website.

Storing the cultures in natural daylight at room temperature should also be fine, providing they are kept out of direct sunlight.

**On receipt of culture:** cultures should be subcultured into fresh sterile medium as described below, ideally within a few days of receipt. If the culture vessel is very full on receipt and subculturing cannot be done immediately, we advise transferring half of the culture to a sterile container to provide air space.

**ACDP Hazard Gp:** 1 - Non pathogenic / non hazardous. Unlikely to cause human disease.

**Culture Medium:** BG11 with *Plectonema* sp. as food (a filamentous cyanobacteria).

**Lighting:** Mix of cool and warm white fluorescent lighting; low/shaded light (in our experience cyanobacteria do not tolerate bright lighting)

**Light Cycle:** 12h light : 12h dark

**Temperature:** 20 degrees C

**Sub Interval:** 4 weeks (will depend on environment)

**Culture Vessel:** 50ml tissue culture flasks with vented caps, laid flat

**Note:** the cyanobacteria food source is grown in BG11 medium and subcultured in the ratio of approx. 1:10 culture: fresh sterile medium every four weeks, stored as above.

### **Culture Method:**

Prepare sterile media and add to sterile tissue culture flasks. Flasks containing the media are stored at 4 degrees C. One hour prior to use the required number of flasks are transferred to 20 degrees C.

To inoculate the fresh media, a dense culture is chosen from existing stocks. The state of the culture is ascertained by microscopical examination. Gently agitate the culture to mix the cells more evenly in the medium and pour about 10ml into a flask of fresh media. Gently agitate the fresh flask and pour about 10ml back into the inoculating flask.

The *Plectonema* is transferred with the ciliate, under normal circumstances there may be no need to add additional food when subculturing, add 0.5ml of *Plectonema* after 14 days.

**Use strict aseptic techniques throughout and if possible carry out all subculturing within a laminar flow cabinet.**