Raphidiophrys ambigua

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.

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: MW (Volvic) plus washed *Tetrahymena* as food source (see below)
Media recipes can be found on our website: www.ccap.ac.uk/index.php/media-recipes/

Lighting: not required

Light Cycle: -

Temperature: 20 degrees C

Sub Interval: 4 weeks (at CCAP, may vary depending on environment); feed weekly

Culture Vessel: petri dishes (approx. 50mm x 18mm)

Culture Method:

Choose three dense cultures from existing stocks. The state of the cultures are ascertained by microscopic examination (x500).

To subculture split each dish into a new petri dish and replenish to approx. 30ml with MW. Then feed with washed *Tetrahymena.*

Raphidiophrys ambigua is fed ⅓ ml washed *Tetrahymena.*

Check culture status weekly and feed as required.

Use strict aseptic techniques throughout and if possible carry out all subculturing within a laminar flow cabinet (particularly important for axenic strains).

Washing *Tetrahymena pyriformis* 1630/1W

This takes about 1 hour to complete and is done every 3 weeks. 1 ml of *Tetrahymena* should be subbed into 200ml flask of PPY three to four days before washing (e.g. sub on Thursday morning for washing on Monday morning). The *Tetrahymena* becomes too dense in the PPY if left for too long and it becomes difficult to wash resulting in more dead cells in the final washed *Tetrahymena*.

You will need: Test tube rack, 4 new centrifuge tubes, 2 sterile 2ml disposable pipettes, 10 ml pipette and electronic *pipette man,* P+J medium, sterile beaker and large unsterile beaker for waste, 8 sterile 50ml flasks.

P+J is a mineral media which does not contain any nutrients (www.ccap.ac.uk/index.php/media-recipes/).

1. Swirl the 200ml flask very gently; Pour 15ml of culture into each of the 4 centrifuge tubes. Balance the tubes with pipette.
2. Centrifuge for 3 mins at 2600-3000 revs.
3. As soon as the centrifuge stops, quickly remove the supernatent down to tapering end using 10 ml pipette. Waste goes into beaker. Leave approx. 2-3 ml in the tubes.
4. Pour P+J from bottle into sterile beaker aseptically. Gently pour or pipette P+J down the side into the centrifuge tubes without disturbing the pellet at the bottom. Leave for 3-5 min.
5. Use disposable pipette to carefully remove pellet from bottom of tube. After the first couple of times there may not always be a pellet to remove. It depends on quality and density of *Tetrahymena* subbed.
6. Use the pipette to mix the cells in each tube (loosen from the sides). Top up to 15ml with P+J. Remember to balance out each tube exactly.
7. Repeat steps 2. to 6. four times. Centrifuging 4 times, move the spare blue capped tube along the spaces in a rack to record how many times the cells have been spun.
8. Ending up with 4 tubes containing 15ml each of cell suspension in P+J.
9. After agitating the liquid to get the cells stuck to the walls of the tubes, Split the contents of each tube into the 50ml flasks and top up with fresh P+J to 25mls. You should now have eight flasks each with 25mls of washed Tetrahymena.
10. Label the flasks with the reference of the culture they are to be fed to (if more than one). This stops cross contamination between the cultures.
11. Place half the flasks at 8°C room and the others at 15°C or cool room temperature. Use the 15°C flasks for feeding first and the 8°C as back up.