Washing Tetrahymena pyriformis

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Protists: Tetrahymena *culture, commonly* T. pyriformis *CCAP 1630/1W*

Apparatus: centrifuge; electronic pipette man.

Cell culture medium: P+J medium (a mineral media which does not contain any nutrients).

Plasticware: 4 new centrifuge tubes; 2 sterile 2ml disposable pipettes; 10ml pipette; sterile beaker; large unsterile beaker for waste; 8 sterile 50ml flasks.

Chemicals used routinely are of Analar grade purchased from Sigma-Aldrich, unless otherwise stated.

PREAMBLE. The methods outlined below are based on those routinely employed at the Culture Collection of Algae and Protozoa (CCAP).

Washed *Tetrahymena* are prepared as food for various amoebae and two other of our strains: *Prodiscophrya collini* and *Raphidiophrys ambigua.* This method takes about one hour to complete, and is carried out every three weeks.

Method

1. 1 ml of *Tetrahymena* should be subcultured into a 200ml flask of PPY medium 3-4 days before washing.

   http://www.ccap.ac.uk/media/documents/PPY.pdf

   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 culture.

2. When ready to wash, swirl the 200ml flask very gently. Pour 15ml of culture into each of the 4 centrifuge tubes, balance the tubes with pipette.

3. Centrifuge for 3 minutes at 2600-3000 revs. As soon as the centrifuge stops, quickly remove the supernatant down to the tapering end using 10ml pipette. Waste goes into the beaker. Leave approximately 2-3 ml in the tubes.

4. Pour P+J into the 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 minutes.

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 the quality and density of *Tetrahymena.*

6. Use the pipette to mix the cells in each tube (loosen from the sides). Top up to 15 ml with P+J. Remember to balance each tube exactly.

7. Repeat steps 3-6 three more times. You will be centrifuging four times. You will end up with four tubes containing 15ml each of cell suspension in P+J.

8. After agitating the liquid to re-suspend 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 8 flasks each with 25mls of washed *Tetrahymena*.

9. The flasks are kept at 15°C in the dark and should last for 3 weeks.

Adjust volumes as necessary if you require more or less final quantity of washed *Tetrahymena.*