**Immunofluorescence of *Crithidia***

1. Count cells of a mid-log phase culture (2-5 x 107 cells/ml)

2. Add 1E7 cells per tube.

3. Spin down all tubes, 5 min, 1000 rcf.

4. Remove all supernatants (leave ~20 μl), Resuspend cell pellets in residual liquid.

5. Resuspend each pellet in 1 ml BHI medium (final cell concentration 1E7 cells/ml)

6. Add 125 μl of 36% formaldehyde to each sample (final concentration 4% formaldehyde).

7. Incubate at room temperature for 5 min. Spin at 1000 rcf for 5 min (10 min fixation total).

8. Remove supernatants leaving ~20 μl behind. Resuspend cells gently in residual liquid.

9. Wash cells 2X with 1 volume (1 ml) of PBS + 0.1 M glycine (add PBS-glycine, spin, remove supernatant, resuspend cells in residual liquid).

10. During spins label slides, draw outlines using Pap pen (2 areas per slide ~2 cm x 2 cm). Prepare humid chamber (place wet paper towel in Ziploc container).

11. After removing supernatant of final wash, resuspend cells in 1 ml PBS.

12. Apply 200 μl of this solution onto coated slides. Cells are allowed to adhere for 10-20 min in a humid chamber.

13. Wash 1X briefly and 1X for 5 min with 50 ml 1X PBS in Coplin jars.

14. Permeabilize in 0.1% Triton X-100 in PBS for 5 min.

15. Wash 2X 5 min in 1X PBS (50 ml each in Coplin jars).

16. Block in blocking solution: PBS + 0.1% Tween-20 + 1% BSA for 1 hour at RT or at 4 °C overnight in humid chamber.

17. At end of blocking step, spin primary antibody in microcentrifuge at 10,000 rpm for 2 min. Prepare antibody dilution by pipetting antibody from the top and adding to the appropriate volume of blocking solution.

18. Remove block. Apply primary antibody in PBS + 0.1% Tween-20 + 1% BSA. Incubate for 1 h at RT or overnight at 4 °C in humid chamber.

19. Wash 3X 5 min in PBS + 0.1% Tween-20 (50 ml each in Coplin jars).

20. During washes spin secondary antibody as in step 17 and dilute in appropriate amount of blocking solution.

21. Remove final wash. Apply secondary antibody in PBS + 0.1% Tween-20 + 1% BSA. Incubate for 30 min-1 h at RT in humid chamber.

22. Wash 3X 5 min in PBS + 0.1% Tween-20 (50 ml each in Coplin jars). During wash steps prepare DAPI solution (1 μl 2 mg/ml stock in 10 ml 1X PBS).

23. Incubate slides in 200 μl 0.2 μg/ml DAPI in PBS for 3 min.

24. Wash briefly in PBS, then 1X with PBS for 5 min (50 ml each in Coplin jars).

22. Mount in 10 μl PBS + 90% glycerol. Seal edges of coverslip with nail polish.