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Paramecium bursaria immunofluorescence protocol

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol has been developed for immunofluorescent localisation of host proteins in *Paramecium bursaria* 186b.

Protocol materials

- Anti-α-Tubulin antibody Mouse monoclonal **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T6199-25UL — Step 8
- CITIFLUOR Antifadent Mounting Medium AF2 **Electron Microscopy Sciences Catalog #**17971-100 — Step 13
- Phosphate buffered saline **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P4417-50TAB — Before starting
- Tween 20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P1379-500ml — Step 5
- Hoechst 33342 **Invitrogen - Thermo Fisher Catalog #**H1399 — Step 11
- Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) **Abcam Catalog #**ab150077 — Step 10
- Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed **Abcam Catalog #**ab150117 — Step 10
- Pierce™ 16% Formaldehyde (w/v) Methanol-free **Thermo Fisher Scientific Catalog #**28906 — Step 3
- Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A2153 — Step 7
- Poly-Prep Slides **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P0425-72EA — Step 14

Before start

Prepare 200 mL of PBS

- Phosphate buffered saline **Sigma Aldrich Catalog #**P4417-50TAB

Fixation

52m

1 Take 6 mL of *P. bursaria* culture per treatment. Centrifuge 800 x g, 00:10:00 and remove 5 mL (i.e. concentrate sample 6-fold). Aliquot 1 mL of concentrated culture into a 1.5 mL micro-centrifuge tube per treatment for downstream steps. 10m

2 Centrifuge 800 x g, 00:10:00 to pellet the cells. Remove supernatant, avoiding the cell pellet. 10m

3 Re-suspend cells in 500 µL PBS containing 4% formaldehyde [375 µL PBS plus 125 µL 16% formaldehyde]. Use a fresh ampule of formaldehyde for each experiment. 12m

Incubate for 00:12:00 Room temperature

Pierce™ 16% Formaldehyde (w/v) Methanol-free **Sigma Aldrich Catalog #**28906

4 Centrifuge 800 x g, 00:10:00 , remove supernatant and wash once in 500 µL PBS. Repeat the centrifugation 800 x g, 00:10:00 20m

Permeabilisation

40m

5 Re-suspend cell pellet in 500 µL PBS containing [M] 0.05 % volume Tween 20. 10m

Incubate for 00:10:00 Room temperature

Tween 20 **Sigma Aldrich Catalog #**P1379-500ml

6 Centrifuge 800 x g, 00:10:00 , remove supernatant and wash **twice** in 500 µL PBS. Repeat the centrifugation 800 x g, 00:10:00 , x2 30m

Blocking

1h

7 Prepare PBS containing [M] 3 % volume BSA. Filter through a 0.2 micron syringe filter prior to use. Re-suspend cells in 500 µL of this solution. 1h

01:00:00 Room temperature

Bovine Serum Albumin **Sigma Aldrich Catalog #**A2153

Primary antibody

16h

8 Add 1 µL primary antibody directly to the cells in PBS+3% BSA. 16h

A good positive control here is 1 µL Anti-α-Tubulin antibody

Anti-α-Tubulin antibody Mouse monoclonal **Sigma Aldrich Catalog #**T6199-25UL

Overnight 4 °C

Note

You will need to titrate an appropriate concentration of the primary antibody here

Secondary antibody

3h

9 After the overnight incubation, wash **three times** in 500 µL PBS. 30m

Repeat the centrifugation 800 x g, 00:10:00 , x3 and re-suspend the cells in 500 µL PBS.

10 Add 1 µL secondary antibody. 2h

For rabbit primary antibodies:

Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) **Sigma Aldrich Catalog #**ab150077

For mouse primary antibodies (e.g. Anti-α-Tubulin antibody used above):

Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed **Sigma Aldrich Catalog #**ab150117

02:00:00 Room temperature in the dark

11 If required, add 2 µL of [M] 10 mg/mL Hoechst 33342 5m

Hoechst 33342 **Sigma Aldrich Catalog #**H1399

00:05:00 Room temperature

12 Centrifuge 800 x g, 00:10:00 , remove supernatant and wash twice in 500 µL PBS. Repeat the centrifugation 800 x g, 00:10:00 , x2 and re-suspend in 20 µL of residual PBS 20m

Preparing slides 25m

13 Add a drop of CITIFLUOR AF2 to the residual PBS. 5m

CITIFLUOR Antifadent Mounting Medium AF2 **Electron Microscopy Sciences Catalog #**17971-100

14 Using a wide-bore tip (i.e. a 1 mL pipette tip with the end cut-off), spot approximately 10 µL onto Poly-Prep Slides **Sigma Aldrich Catalog #**P0425-72EA 5m

> **Note**
>
> Add only one sample to the centre of each slide to allow for sealing

15 Add a coverslip and seal with clear nail polish. 15m