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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

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

Before start

Prepare 200 mL of PBS

- ✕ Phosphate buffered saline **Merck MilliporeSigma (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 μ L PBS containing 4% formaldehyde [375 μ L PBS plus 125 μ L 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 Thermo Fisher Scientific Catalog #28906

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

Permeabilisation

40m

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

Incubate for 00:10:00 Room temperature

Tween 20 Merck MilliporeSigma (Sigma-Aldrich) Catalog #P1379-500ml

- 6 Centrifuge 800 x g, 00:10:00 , remove supernatant and wash **twice** in 500 μ L 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 μ L** of this solution.

1h

01:00:00 **Room temperature**

Bovine Serum Albumin Merck MilliporeSigma (Sigma-Aldrich) Catalog #A2153

Primary antibody

16h

- 8 Add **1 μ L** primary antibody directly to the cells in PBS+3% BSA.

16h

A good positive control here is **1 μ L** Anti- α -Tubulin antibody

Anti- α -Tubulin antibody Mouse monoclonal Merck MilliporeSigma (Sigma-Aldrich) Catalog #T6199-25UL

Overnight **4 $^{\circ}$ 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 μ L** PBS.

30m

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

- 10 Add **1 μ L** secondary antibody.

2h

For rabbit primary antibodies:

Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) Abcam Catalog #ab150077



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



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



02:00:00



Room temperature in the dark

- 11 If required, add  2 μ L of [M] 10 mg/mL Hoechst 33342

5m



Hoechst 33342 **Invitrogen - Thermo Fisher Catalog #H1399**



00:05:00



Room temperature

- 12 Centrifuge  800 x g, 00:10:00 , remove supernatant and wash twice in  500 μ L PBS. Repeat the centrifugation  800 x g, 00:10:00 , x2 and re-suspend in  20 μ L 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 μ L onto

5m



Poly-Prep Slides **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P0425-72EA**

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

