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# Paramecium bursaria immunofluorescence protocol

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# OPEN ACCESS



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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.

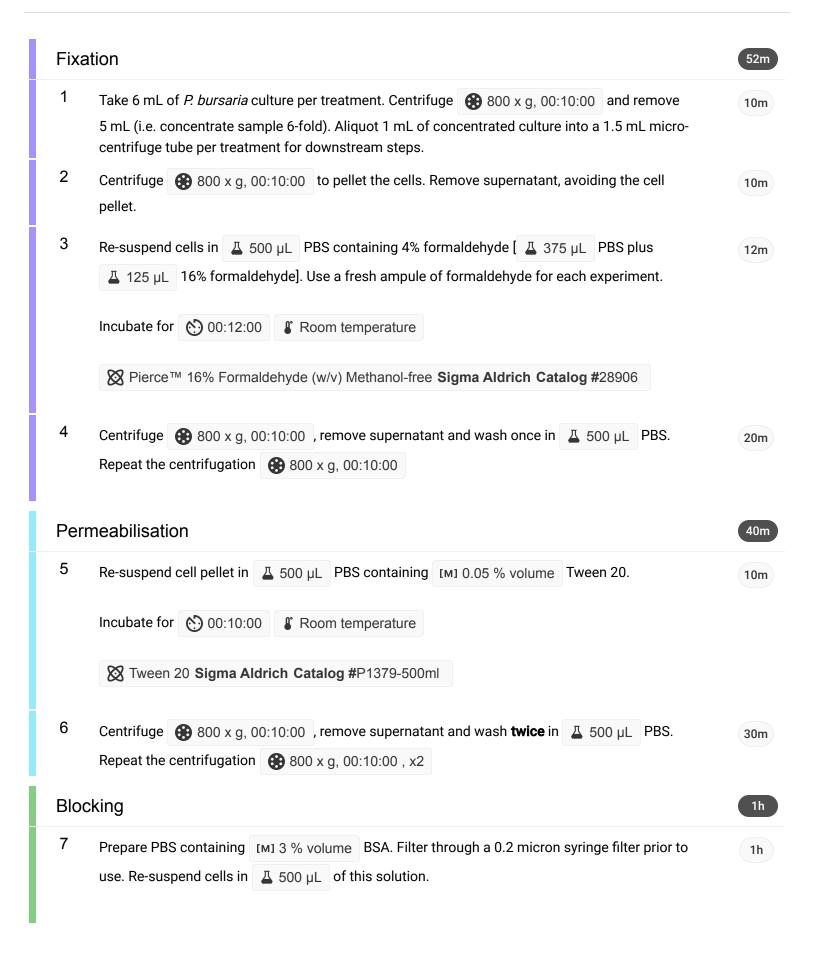
## Protocol materials

X Anti-α-Tubulin antibody Mouse monoclonal Merck MilliporeSigma (Sigma-Aldrich) Catalog #T6199-25UL	Step 8
Phosphate buffered saline Merck MilliporeSigma (Sigma-Aldrich) Catalog #P4417-50TAB  Before starting	
Tween 20 Merck MilliporeSigma (Sigma-Aldrich) Catalog #P1379-500ml Step 5	
Moechst 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 <b>Abcam Catalog #</b> ab150117 Step 10	
<b>⊠</b> Pierce <sup>™</sup> 16% Formaldehyde (w/v) Methanol-free <b>Thermo Fisher Scientific Catalog #</b> 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







♦ 01:00:00 S Room temperature

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

## Primary antibody

16h

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

16h

A good positive control here is  $\perp \!\!\!\! \perp 1 \mu L$  Anti- $\alpha$ -Tubulin antibody

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



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  $\Delta$  500 µL PBS.

30m

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



