Paradise Fish Phalloidin Staining Protocol

Szabó et al. (2025) Biologia Futura

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1 Protocol Overview

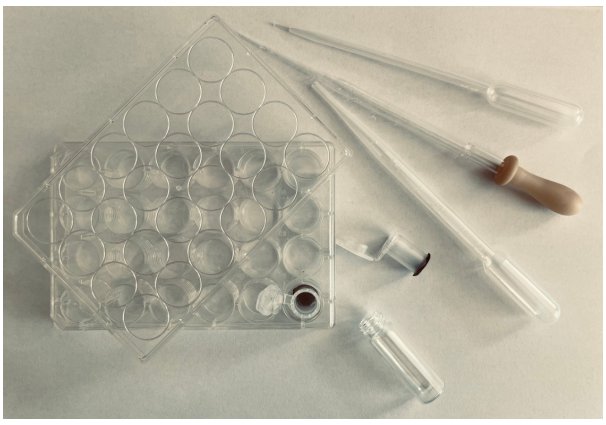
Step	Duration	Temperature	Notes
Fixation	Overnight	4°C	4% PFA in PBS
Sample Handling	~5 min	RT	Use 24-well plates with strainers
PBST Washes	3 × 5 min	RT	Standard washing
Permeabilization	1.5 hours	RT	2% PBTr
Phalloidin Staining	2 hours	RT	1:1000 dilution, protect from light
Post-staining Washes	2 × 30 min	RT	2% PBTr
Microscopy	Variable	RT	Appropriate fluorescence settings

2 Equipment and Materials

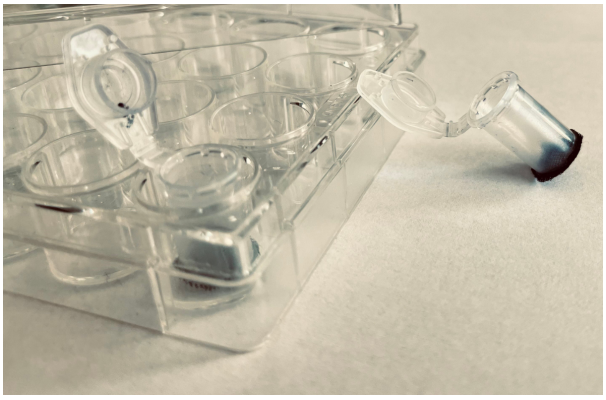
Materials	Equipment
24-well plate	shaker
strainers (DIY)	fluorescence microscope
glass pipettes (wide bore)	
pasteur-pipettes (3 mL)	
parafilm	
aluminium foil	

! Making DIY Stainers

We created our stainers by gluing the upper part of 2 mL Eppendorf tubes to a piece of mosquito net with the mesh size of 0.2 x 0.4 mm.



(a) Plasticware used in this protocol.



(b) DIY stainers.

Figure 1: Materials used in this protocol.

3 Solutions and Reagents

Reagents & Suppliers

Primary Reagents and Suppliers

Reagent	Supplier	Catalog.Number
Phalloidin	Abcam	ab176753
Triton X-100	Reanal	20340
Tween-20	Sigma	P1379-100ml

> **Warning**
>
> These suppliers and catalog numbers are only indicative. Equivalent reagents from other suppliers will probably work equally well.

Buffer Preparation

10× Phosphate Buffered Saline (PBS) Stock (1 L)

Component	Amount
NaCl	80 g
KCl	2 g
Na_2PO_4	14.4 g
KH_2PO_4	2.4 g
Distilled water	Fill to 1 L

> **10x PBS Stock Note**
>
> Adjust pH to 7.4 and autoclave.

> **Storage**
>
> Store at room temperature (RT).

1× PBS

Component	Amount
10× PBS	1 part
Distilled water	9 parts

Storage

Store at room temperature (RT).

PBS with Tween-20 (PBST) (50 mL)

Component	Amount	Final concentration
1× PBS	Fill to 50 mL	-
Tween-20 (20% stock)	250 µL	0.1%

Tween-20 Tip

Tween-20 is highly viscous. Prepare a 20% Tween-20 stock solution (dilute pure Tween-20 1:5 in distilled water) for easier pipetting.

Storage

Store at RT.

2% PBS with Triton-X (PBTr) (50 mL)

Component	Amount
25% Triton X-100 stock	4 ml
1× PBS	Add to 50 ml total

Triton-X Tip

Triton-X is highly viscous. Prepare a 25% Triton-X stock solution (dilute pure Triton-X 1:4 in distilled water) for easier pipetting.

Storage

Mix well after preparation. Store at RT.

4 Staining Procedure

Step 1: Fixation

- Fix samples in **4% paraformaldehyde in PBS**
- Incubate **overnight at 4°C**

Step 2: PBST Washes

Transfer embryos into strainers

Transfer embryos or samples to 24-well plates with strainers. This step is essential as due to the presence of the lipid droplet, embryos will float .

Solution	Duration	Temperature
PBST	3 × 5 min	Room temperature (RT)

Step 3: Permeabilization

Solution	Duration	Temperature
2% PBTr	1.5 hours	RT

Step 4: Phalloidin Staining

Solution	Duration	Temperature
2% PBTr + phalloidin (1:1000 dilution)	2 hours	RT

Warning

Light Protection Required: Wrap samples in aluminum foil during phalloidin incubation to prevent photobleaching.

Step 5: Post-staining Washes

Solution	Duration	Temperature
2% PBTr	2 × 30 min	RT

> ⚠ Warning
>
> **Light Protection Required:** Wrap samples in aluminum foil during post-staining washes to prevent photobleaching.

Step 6: Microscopy

- Proceed to **microscopy** using appropriate fluorescence settings.

5 Results

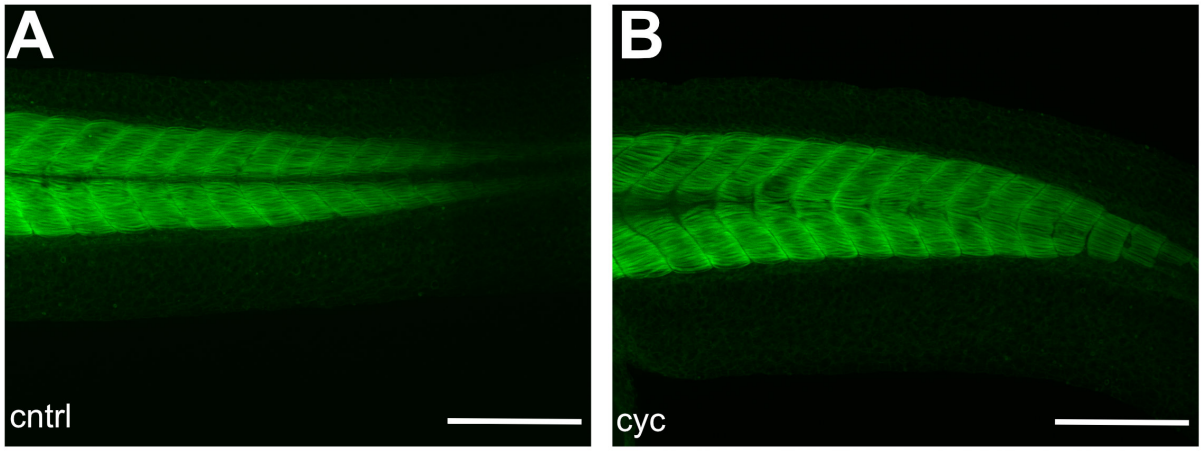


Figure 2: Control (A) and cyclopamin treated (B) paradise fish samples stained with phalloidin.

6 Troubleshooting

Common Issues and Solutions

Problem	Possible Cause	Solution
Weak staining	Phalloidin degradation	Use fresh phalloidin, protect from light
High background	Insufficient washing	Increase wash duration or frequency
Poor penetration	Inadequate permeabilization	Extend permeabilization time
Photobleaching	Light exposure	Keep samples in dark, use anti-fade mounting