Zebrafish Immunofluorescence Protocol

Kim Kobar[1]

[1]University of Wisconsin-Superior

Apr 08, 2019

Working

dx.doi.org/10.17504/protocols.io.zwif7ce

Kim Kobar

MATERIALS

NAME	CATALOG #	VENDOR
DMSO	BP231	Fisher Scientific
Goat Serum	16210-064	Gibco - Thermo Fischer
OmniPur BSA Fraction V (Bovine Serum Albumin) Heat Shock Isolation	2910-25GM	
Triton X-100	BP151-100	Fisher Scientific
DPBS 10X with Calcium and Magnesium	14-080-055	Gibco - Thermo Fischer

MATERIALS TEXT

Recipes

IF Block solution

1. To make 10mL of blocking solution, combine and vortex the following in a 15mL conical:
2. 200µL goat serum (freezer)
3. 0.1g Bovine Serum Albumin (fridge)
4. 100µL DMSO (flammable cabinet)
5. 100µL 10% Triton X-100 (cabinet)
6. 1mL 10X PBS (cabinet)
7. 8.6mL DI H2O
8. Solution should be cloudy, but if there are visible chunks floating around, place in 37°C water bath until uniform solution.
9. Store at 4° for 1 week or store at -20° for long term.

1X PBS

1. Add 9.6g of HyClone PBS (cabinet) to 1L of DI H2O
2. Stir until dissolved and store at RT

PBS - 0.1% Triton

1. Add 100µL of Triton (cabinet) to 100mL 1X PBS

PBS - 2% Triton X-100

1. Add 2mL of Triton (cabinet) to 100mL 1X PBS

Day 1

1 Dechorionate embryos with two pairs of forceps.

Under a dissecting microscope, pinch an embryo's chorion using a pair of forceps held in one of your hands. With the forceps in your other hand, pinch the chorion near to the original pinch and gently tear the chorion by separating your hands. Repeat pinching and tearing chorions with forceps until the embryos are dechorionated.

2 Using a large bore pipette, transfer dechorionated embryos into labelled microcentrifuge tubes, up to 50 embryos/tube.

The labels should include: # of embryos, age in hpf, what was injected into them & how much, and the date the embryos were injected. Label the top and side of the tube.

3 Remove as much liquid as possible from the microcentrifuge tubes with a small bore pipette.

4 Fix dechorionated embryos in ~1mL of 4% PFA (if used every week - stored in 4°C; new aliquots are stored long term at -20°C for 1 hour at RT on the rocker). Tubes should be placed on their side whenever they are rocking. Orient the tubes perpendicular to the rocking motion such that the embryos rock from side to side in the tube rather than from cap to bottom.

SAFETY INFORMATION

Wear gloves when working with PFA

5 Discard PFA from tubes into PFA waste using a small bore pipette. Take care to not pipet or damage any embryos when removing solutions.

6 Rinse in ~1mL PBS 2 x 5 mins at RT on the rocker. Remove solution with a small bore pipette each time before adding new solution.

00:05:00 **First Wash**

00:05:00 **Second Wash**

Embryos can then be stored in fresh PBS for 2 weeks max at 4°C (in the box labeled “Fixed Embryos” - be sure to add each tube to the “Fixed Embryos Log.”

START HERE when using previously fixed embryos:

7 **For 24-48 hpf embryos:**
-wash in ~1mL DI H2O for 5 min at RT on the rocker.
For ~72 hpf embryos:
-wash 5 min in DI H2O at RT on the rocker
-put in 100% acetone at -20°C for 5 min,
-then wash in DI H2O again for 5 min at RT on the rocker.

8 Incubate for 1 hour in 500 ul IF Block solution at RT on the rocker.

IF Block is stored in 15 ml conicals in the -20°C - however, keep IF Block at 4°C from now until done making the 2° Antibody Solution on Day 2 (this is to avoid repeated freeze/thaw cycles).

9 Remove IF Block solution from embryos and discard in waste beaker.

10 Replace with 1° Antibody solution: 100 ul per tube of embryos (1° antibody is diluted in IF Block solution, the amount of dilution varies with each antibody - see the table at the end of this protocol to determine correct concentration).

11 Incubate overnight at 4 °C

Day 2

12 Remove the 1° Antibody Solution and discard in waste beaker.

13 Rinse all day in 100µL-500µL PBS- 0.1% Triton at room temperature on the rocker.

Change the solution 3-4 times throughout the day, ensuring to remove solution before adding new solution.

14 Remove PBS-0.1% Triton and replace with 2° Antibody Solution.

Choose the Dylight488 (green) conjugated 2° antibody made against the species in which your primary antibody was produced. **However, if doing a dual stain, choose Dylight 594 (red).** Secondary antibodies are diluted at 1:750 in IF block (see table): 100 ul per tube of embryos.

15 Incubate **in dark** (wrap with tin foil) overnight at 4°C.

Keep in dark as much as possible from now on out.

Day 3

16 Remove solution in tubes with small bore pipette. Rinse in PBS- 0.1% Triton 2 x 5min at RT on the rocker.

17 Store in at least 200 ul PBS at 4°C.

Try to image within a week to avoid degradation.

Antibody Dilution Table

18 Primary Antibody Dilutions: prepare ~100 ul per tube of 50 embryos
Most primary antibodies are stored in the "Research S18" box in 4C fridge.

Example: 1 = 5 ul antibody
20 95 ul IF Block

Name	Dilution	Species	Vendor/Product #
F310	1:20/1:50	mouse	
A4.1025	1:20	mouse	
Phalloidin (Actin)	1:20		
PH3	1:500	rabbit	
CC3	1:500	rabbit	
Dylight 488 α-rabbit	1:750		
Dylight 488 α-mouse	1:750		

Dylight 594 α-rabbit	1:750		
Dylight 594 α-mouse	1:750		
HRP α-rabbit			
HRP α-mouse			