

Worm Antibody Staining

Reagents Needed:

M9 (common stock)	AbA
formaldehyde solution	primary antibody
1X PBS-Tween (pH 7.2)	secondary antibody
Tween-20 (optional)	additional dyes (optional)
b-ME solution	mounting media
collagenase solution	

Procedure:

I. Fixing Worms

1. Grow up worms on OP50 plates (the size and number of plates depends on how many worms you need but in general one big plate for a time point is more than enough).
2. When the plates are the right stage, but not starved, wash the worms off with M9 and put them in Eppendorf tubes.
3. Spin the worms for 1-2 min at 1000xg, remove the liquid, and wash again with M9 (common lab stock in worm room).
4. Repeat step 3 two more times.
5. Put Eppendorf tubes on ice for a few minutes to cool down. Remove excess liquid and add 500 μ L ice-cold formaldehyde solution.
6. Incubate worms for 15-30 minutes at room temperature.
7. Wash fixed worms 3 times in 1X PBS-Tween (pH 7.2) using the same procedure as in step 3.
8. At this point animals can be kept at 4°C for a few weeks.

*Optional: Permealization Treatment (if staining is poor)

1. Incubate with 2% Tween-20 in 1X PBS (pH 7.2) for 30 minutes at room temperature.
2. Wash 3 times with 1X PBS-Tween (pH 7.2).

II. b-ME Treatment

1. Spin worms and remove most of the supernatant without disturbing the pellet.
2. *In the hood*, resuspend the pellet in 1mL of b-ME solution.
3. Incubate the worms at 37°C overnight on a nutator mixer to allow gentle movements.
4. Wash 3 times in 1X PBS-Tween (pH 7.2), doing the first wash in the hood. Incubate between each wash with gentle mixing (~1 hour at room temperature or overnight at 4°C).
5. Resuspend the worms in 50-100 μ L of 1X PBS-Tween (pH 7.2).
6. Again, at this point the worms can be kept at 4°C for a few weeks.

* For Phalloidin staining you can jump directly to the antibody staining procedure.

III. Collagenase Treatment

1. Transfer about 10 μ L of worms to a 1.5 mL Eppendorf tube. Or use the whole tube, spinning down the worms and removing most of the PBS-Tween.
2. Add 100-150 μ L of collagenase solution to each tube.
3. Incubate at 37°C with violent shaking (~4000 rpm).
4. Check the tube frequently starting after about 5 minutes, depending on your samples. When 20% of the worms are broken, stop the reaction by placing on ice.
5. Wash the worms 2 times with 1X PBS-Tween (pH 7.2) and 1 time with AbA.
6. Worms can be stored at 4°C.

IV. Antibody Staining

1. Spin worms (as above) and resuspend in 200 μ L of AbA.
2. Add your primary antibody 1:100 -1:1000.
3. Incubate samples with rocking overnight at room temperature. It is suggested to incubate for additional time at 4°C in case the antibody binds better at this temperature.
* Some antibodies may bind better at different temperatures.
4. Wash worms 3 times with AbA, incubating between spins with rocking for 1-2 hours at room temperature. Can leave overnight at 4°C.
5. Add 200 μ L of AbA containing your secondary antibody 1:100. Keep samples in the dark and incubate with rocking for a few hours at room temperature. Can leave overnight at 4°C.
6. Wash worms 3 times with AbA, incubating between spins with rocking for 1-2 hours.
*Optional: If you wish to stain with additional dyes, add the dye to 200 μ L AbA
7. (example- DAPI 2mg/mL, Phalloidin 1:100) with rocking for up to 1 hour. Then wash worms 3 times with AbA, incubating between spins with rocking for 1-2 hours. Can leave overnight at 4°C.
8. Remove all liquid but 10 μ L and add 10 μ L of mounting media to each tube.
9. Using a tip with the end cut-off, mount 5-10 μ L of worms on a slide. Top with a cover slide and seal with nail polish. If slide cannot be used immediately, store at -20°C.

Recipes:

Formaldehyde Solution

1.0g paraformaldehyde into 12.5 mL dH₂O

- Heat to 55-60°C in water bath add 2-4 drops of 1M NaOH.
- Heat until solution clears.
- Cool solution on ice and add 12.5 mL 0.2M PO₄ pH 7.2.
- Store at -20°C until needed.

Collagenase Solution (600 μ L)

480 μ L dH₂O

120 μ L Tris (pH 7.4)

0.6 μ L CaCl₂

12 μ L Collagenase (stored at 4°C)

(C-5138 100mg Collagenase IV Sigma in 1mL of 0.1M Tris pH 7.4)

NOTE: Collagenase will vary from stock to stock. First three ingredients can be combined and kept as a stock solution at room temperature. Simply add the Collagenase right before use.

M9 (1L)

* Common lab stock in worm room.

5.8g Na₂HPO₄•7H₂O

3.0g KH₂PO₄

5.0g NaCl

0.25g MgSO₄•7H₂O

ddH₂O to 1L

• Filter (0.22 μ m) and bottle.

b-ME Solution

* Add b-ME in the hood!

1ml dH₂O

400 μ L 0.5M Tris (pH 6.8)

15 μ L Triton X-100

76 μ L b-ME

1X PBS-Tween (pH 7.2) (500mL)

25mL 20X PBS (pH 7.4)

475mL dH₂O

250 μ L Tween-20

AbA (~40mL)

38mL dH₂O

2mL 20X PBS

200 μ L Triton X-100

0.4g BSA

Mounting Media (100mL)

10 μ L 1X PBS (pH 7.2)

0.1 mg/mL DABCO (add DABCO to PBS first)

90 μ L glycerol

References:

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