

Gonad/Intestine Immuno-Staining by Barth Grant

Adapted From R. Francis, Schedl Lab and Sarah Crittenden, Kimble Lab by Barth Grant.

1) Pick adults and/or L4s to an unseeded NGM plate. Alternatively, wash worms off a seeded plate with 1.5 ml of egg buffer and spin down in an eppendorf tube, then wash once with 1.5 ml of egg buffer. Transfer as many worms as can be dissected in 15 min (~200). If we are going to prepare multiple batches of dissected gonads/intestines we hatch bleached eggs in M9 buffer, then aliquot them to plates to grow up as a synchronized population (about 200 worms/plate). L1 progeny of these worms present during dissection do not interfere with anything.

2) Suspend washed worms from one plate in 0.2-0.3 ml of egg buffer + 0.2 mM levamisole and transfer to a glass dish. Depression slides work well. Use the deepest you can find.

3) As paralysis sets in, begin cutting off heads at level of the pharynx. Place the head between two 25 gauge syringe needles and decapitate by moving needles in a scissors motion (avoid needles with bent tips). For most animals, at least one gonad arm and the intestine should extrude completely.

4) Transfer cut worms with a 100 ul mouth pipette to a presiliconized 1.7 ml epp tube (I buy "Slickseal" tubes from National Scientific).

5) Fix the cut worms by adding 1 ml of 1.25% freshly made paraformaldehyde in egg buffer. Rock at RT for 10 min. Some people post fix in -20°C methanol for 5 min. Cut worms can be stored up to two weeks in -20°C methanol. We haven't tried the methanol post fix ourselves. GFP autofluorescence survives the aldehyde fix well, but probably not the methanol.

6) Preincubate in 1 ml PTB at RT for at least one hour. Change buffer at least 2X. Cut worms are spun down for 30 sec to 1 min at 4,000 rpm between buffer changes. Worms can be stored at 4°C for a few days in PTB. The tween20 in PTB appears to very quickly abolish fluorescence of autofluorescent gut granules (lipofucin), but has no effect on GFP.

7) Spin down cut worms, remove supe, add primary Ab diluted in PTB. Incubate 4 hours at RT or overnight at 4°C with rocking.

8) Wash for 1-4 hours at RT in PTC (four buffer changes, 1 ml each).

9) Preincubate in PTB(+ optional 10% serum) at RT for 30 min.
Incubate in secondary antibody diluted in PTB for at least two hours at RT. We often use 1:500 dilutions of Cy3 or Alexa 488 conjugated antibodies from Jackson Immunoresearch or Molecular Probes.

10) Wash for 1-4 hours at RT in PTC (four buffer changes, 1 ml each). You can never wash too much. An extra overnight wash at 4°C can give nice results.

11) Spin down cut worms, remove supe, resuspend in a glycerol antifade reagent. We like Slowfade Light from Molecular Probes. Store at -20°C. Mount on pads of Permanent Springtime Agarose just before viewing. Can seal with nail polish. Slides don't last long, but can be stored briefly at 4°C.

Egg Buffer:

NaCl (118 mM)

KCl (48 mM)

CaCl₂•2H₂O (2 mM)

MgCl₂•6H₂O (2 mM)

Hepes (25 mM)

Adjust pH to 7.3 with 1N NaOH.

Filter Sterilize

PTC:

0.1% BSA

1X PBS

0.1% Tween20

0.05% Na Azide

1mM EDTA

PTB:

1% BSA

1X PBS

0.1% Tween20

0.05% Na Azide

1mM EDTA

(Can also add 10% serum for preincubations, e.g. Goat serum if using a goat secondary).

Permanent Springtime Agarose:

50 mM Tris Cl pH 9.5

5 mM MgCl
2% agarose

Fresh 1.25% Paraformaldehyde/PBS:

Mix 0.625g powdered paraformaldehyde with 25ml dH₂O and 1-2 drops 1M NaOH. Heat to 65°C. Mix until dissolved. Add dilute HCl until pH drops to between 6 and 8 as judged by pH paper. Add 5 ml 10X PBS. Bring up to 50ml final volume with dH₂O. Use the same day.

Fresh dilutions of concentrated EM grade formaldehyde from sealed ampules is also acceptable.