

L1 Arrest Survival Assay

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1. Pick 25-50 L4 larvae or young adults to each 10 cm plate. Grow for 3-5 days until gravid. Note age of plates, bacteria, temperature, humidity, etc.
2. Wash gravid worms off plates with M9 into 15 ml conical tube using glass serological pipettes and/or Pasteur pipettes. Avoid chunks of agar as they will carry through the procedure. Do not use plates with starved animals.
3. Pellet worms by gentle spinning for 30 seconds in a clinical centrifuge at 1500 rpm.
4. Remove supernatant leaving as little solution behind as possible.
5. Add 6 ml bleach solution.
6. Vortex until all of the worms have dissolved. The total time varies depending on the age and temperature of the bleach and the number of worms. Watch the tubes carefully! Bleaching too long will drastically decrease survival of the embryos.
7. Pellet embryos by spinning at 1500 rpm in the clinical centrifuge for 1 minute.
8. Remove as much supernatant as possible without disturbing pellet by decanting the bleach solution into the sink.
9. Immediately add 10 ml M9 or S medium for wash. Invert the tube a couple of times.
10. Pellet embryos by spinning at 1500 rpm in the clinical centrifuge for 1 minute.
11. Remove supernatant by decanting. Add another 10 ml M9 or S-basal for a second wash. Invert the tube a couple of times.
12. Pellet embryos by spinning at 1500 rpm in the clinical centrifuge for 1 minute.
13. Remove supernatant, and resuspend embryos in a small volume by flicking/vortexing the tube. I use 1 mL for every two 10 cm plates.
14. Determine the titer of embryos by pipetting 1-3 μ L of embryo solution five times to an unseeded plate and counting the number of embryos in each spot. Determine the mean. Also, observe the embryos. The embryos should be free of carcasses (dead dauers are most common), mostly young (free of 2- and 3-fold embryos), and there should be very few embryos that have darkened or lost morphology during the bleaching procedure (these will not hatch).
15. Resuspend the embryos at 1 embryo per μ L in 5 mL of M9 or S medium in a 16 mm glass test tube. Starvation survival depends on density, with higher densities surviving longer, and buffer, with larvae surviving longer in S medium than M9. Consistent densities and culture volumes will improve reproducibility.
16. Culture the tube on a tissue culture roller drum at 20°C.
17. Remove 1 mL aliquots of the culture every three days and score survival. Survival can be scored either by direct observation of the starved larvae or by feeding them (transfer to plates) and scoring them after 2-5 days for the ability to recover and develop to adulthood. Scoring recovery/development is better in the sense that it is what matters, but it is also a bit subjective, because you will have to decide when to score after feeding. The worse off they get while starved, the slower and more variable recovery becomes. Fast recovering animals will begin to lay eggs that hatch making it difficult to distinguish first and second-generation larvae.

Reagents:

Bleach solution (it lasts at 4°C for a couple of months)

Per 10 mL:

- 2 ml 6% NaOCl (Fisher, SS290-1)
- 1 ml 5 M NaOH
- 7 mL ddH₂O