

EGG PREP PLATES (will yield age-synchronized adult worms for toxicity testing)

1. Use several plates containing high concentration of eggs (>5000). These can be 3-day old dauer plates, or any other plates with adequate numbers of eggs. Using considerable pressure on a wash bottle, apply K-medium, then gently rub entire surface of plates with flame-smoothed glass probe to loosen eggs.
2. Transfer all suspended worms and eggs into 4-6 1.5 ml centrifuge tubes using a pipette. Repeat step 1 to ensure complete recovery of all eggs.
3. Balance tubes and centrifuge at about 3000rpms for 7 min to pellet everything.
4. Remove supernatant (use sterile pipette) from all tubes and re-suspend pellet of one tube in about 10 ml clorox solutions. Transfer to one of the remaining pellets, re-suspend, and transfer to the next, etc. until all pellets are consolidated in one tube. Let it sit for 4 min. Re-suspend pellet with pipette every few minutes. (Clorox will kill all worm stages except the eggs.) Do not let sit more than the 10 min or the egg prep will yield fewer eggs.
5. Centrifuge at about 2000 rpms for 7 min to pellet and remove the supernatant. You may continue to use the same pipette because you will be diluting the Clorox solution.
6. Re-suspend pellet in K- medium.
7. Repeat steps 5 and 6 two more times for a total of 2-3 K- medium rinses to thoroughly wash the pellet.
8. Remove supernatant until about 0.5 ml remains. Re-suspend and transfer one drop onto a K+O plate with bacterial lawn. Examine the concentration of eggs under a dissecting microscope. Add enough drops to ensure that each plate has about 400-600 eggs. Incubate at 20°C until desired development stage has been reached (see chart); in most cases this will be 3-4 days to obtain age-synchronized adult worms for testing.
9. With ideal egg prep, 2 egg plates should yield enough worms to set up a toxicity test using 2-3 Costar 12-well culture trays.

Developmental stages from egg hatch in the presence of food (OP50) according to Cassanda and Russell's 1975 Dev. Biol. paper. The developmental cycle at 20 °C:

- a. Eggs laid at 0hrs.
- b. Egg hatches after 10-12 hrs.
- c. First molt lethargus (L1) 26hrs.
- d. Second molt lethargus (L2) 34-36hrs.
- e. Third molt lethargus (L3) 42-45hrs.
- f. Fourth molt lethargus (L4) 56hrs.

g. Egg laying begins (Adult) at ~65hrs

To make 10 ml of Clorox Solution:

2.5 ml of 1M NaOH

1.75 ml of Clorox

5.75 ml of H₂O