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Culturing *C. elegans* worms in liquid culture

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1 Works for me dx.doi.org/10.17504/protocols.io.5v9g696


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ABSTRACT

Growing *C. elegans* worms in liquid culture

MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
100000-1000000 arrested L1 worms	View	
S Basal solution	View	
1 M Potassium Citrate	View	
Trace metals solution	View	
1 M Calcium Chloride	View	
OP50 pellets	View	
conical bottom centrifuge bottles	View	

- Copy-pasted protocol from the Wormbook. Got a protocol from Asia Kosalka. ☐ [liquidcultureAsia.docx](#) Added my notes below.
- Reagents*
- S Basal [5.85 g NaCl, 1 g K₂ HPO₄, 6 g KH₂PO₄, 1 ml cholesterol (5 mg/ml in ethanol), H₂O to 1 litre. Sterilize by autoclaving.]
- 1 M Potassium citrate pH 6.0 [20 g citric acid monohydrate, 293.5 g tri-potassium citrate monohydrate, H₂O to 1 litre. Sterilize by autoclaving.]
- Trace metals solution [1.86 g disodium EDTA, 0.69 g FeSO₄•7 H₂O, 0.2 g MnCl₂•4 H₂O, 0.29 g ZnSO₄•7 H₂O, 0.025 g CuSO₄•5 H₂O, H₂O to 1 litre. Sterilize by autoclaving. Store in the dark.]
- 1 M CaCl₂ [55.5 g CaCl₂ in 1 litre H₂O. Sterilize by autoclaving.]
- S Medium [1 litre S Basal, 10 ml 1 M potassium citrate pH 6, 10 ml trace metals solution, 3 ml 1 M CaCl₂, 3 ml 1 M MgSO₄. Add components using sterile technique; do not autoclave.]

- 8 4 large plates of *C. elegans*, just cleared of bacteria
- 9 concentrated *E. coli* OP50
- 10 *Methods*
- 11 Add 250 ml S Medium to a sterilized 1-2 litre flask.
- 12 Inoculate the S Medium with a concentrated *E. coli* OP50 pellet made from 2-3 litres of an overnight culture.
- 13 Wash each of 4 large plates of *C. elegans* (just cleared of bacteria) with 5 ml S Medium and add to the 250 ml flask.
- 14 Put the flask on a shaker at 20°C. Use fairly vigorous shaking so that the culture is well oxygenated.
- 15 Cultures should be monitored by checking a drop of the culture under the microscope. If the food supply is depleted (the solution is no longer visibly cloudy) add more concentrated *E. coli* OP50 suspended in S Medium. When there are many adult animals in each drop, the culture is ready to be harvested. This is usually on the 4th or 5th day.
- 16 Put the flask on ice for 15 minutes to allow the worms to settle.
- 17 Aspirate most of the liquid from the flask.
- 18 Transfer the remaining liquid to a 50 ml sterile conical centrifuge tube and spin for at least 2 min at 1150 × g to pellet the worms. Young larvae may take longer than 2 min to pellet.



Conical bottom plastic centrifuge bottle

- 19 Aspirate the remaining liquid.
The worms harvested from growth in liquid culture are usually longer and thinner than those grown on petri plates, and tend to hold their eggs. The number of adults, larvae and eggs obtained depends on the strain of worms used, the amount of bacteria provided and the length of time the culture is grown. Freshly cleared cultures will yield worms equal to half the weight of the bacteria used ([Lewis and Fleming, 1995](#)).

Notes

- 20 Add arrested L1 worms to the liquid culture. This has several benefits:
- minimises contamination
 - synchronised growth which means that the bacterial depletion will occur at a more predictable rate



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