OCT 16, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.xcpfivn

Protocol Citation: Vidur Sabharwal 2019. Setting up a liquid culture and harvesting C.elegans. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.xcpfivn>

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Protocol status: Working
 We use this protocol and it's working

Created: Jan 22, 2019

Last Modified: Oct 16, 2019

PROTOCOL integer ID:
 19567

Setting up a liquid culture and harvesting C.elegans

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MATERIALS

S Basal [5.85 g NaCl, 1 g K₂HPO₄, 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 just before use, using sterile technique; do not autoclave.]

IP buffer [15mM HEPES pH 7.6, 10mM KCl, 1.5mM MgCl₂, 0.1mM EDTA, 0.5mM EGTA, 44mM Sucrose, 100mM NaCl.

Sterilize by autoclaving

Add the following components before use, 1x Protein Inhibitor cocktail from 25x stock (Sigma cat no. #11873580001), 30mM NEM from 300mM(0.1g in 2.67ml) stock in ethanol(Sigma cat no. #E3876)]

1 Transfer 20 L4 hermaphrodites each to 10 90cm plates. They will starve out in approximately 6

days. Start the following steps on the day the plates are almost starved. 🌡️ 20 °C

🕒 144:00:00

- 2 Add 30ml S Medium to a sterilized 250 ml flask.
- 3 Inoculate the S Medium with a concentrated *E. coli* OP50 pellet to a final concentration of 30g/L
- 4 Wash each of 5 large plates (90cm) of *C. elegans* (just cleared of bacteria) with 5 ml S Medium and add to the 30 ml flask.
- 5 🕒 84:00:00 🌡️ 20 °C
Put the flask on a shaker at 200rpm
- 6 Put the flask on ice to allow the worms to settle. 🕒 00:15:00
- 7 Aspirate most of the liquid from the flask.
- 8 Transfer the remaining liquid to a 15 ml borosil conical tube using a glass pasteur pipette and spin for at least 2 min at 500 × g to pellet the worms. Young larvae may take longer than 2 min to pellet. 🕒 00:02:00
- 9 Aspirate the remaining liquid and wash with 10x volume of M9 twice. Spin for at least 2 min at 500 × g to pellet the worms

Step 9 includes a Step case.

No Contamination found

Contamination found

step case

No Contamination found

In case of no contamination the worms will be separated from OP50 since OP 50 does not settle at 500xg

- 10** Transfer the worms to a 1.5ml eppendorf, incubate on ice for 10 mins 00:10:00 and remove as much supernatant as possible.
- 11** Add IP Buffer to the Pellet with 2 parts homogenization buffer : 1 part worm pellet and store at -80C for upto 3 months