Setting up a liquid culture and harvesting C.elegans

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1 Works for me

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MATERIALS TEXT

S Basal [5.85 g NaCl, 1 g K_2 HPO_4, 6 g KH_2PO_4, 1 ml cholesterol (5 mg/ml in ethanol), H_2O 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, H_2O to 1 litre. Sterilize by autoclaving.]

Trace metals solution [1.86 g disodium EDTA, 0.69 g $FeSO_4$ •7 H_2O, 0.2 g MnCl2•4 H_2O, 0.29 g $ZnSO_4$ •7 H_2O, 0.025 g $CuSO_4$ •5 H_2O, H_2O to 1 litre. Sterilize by autoclaving. Store in the dark.]

1 M $CaCl_2$ [55.5 g $CaCl_2$ in 1 litre H_2O. 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 $CaCl_2$, 3 ml 1 M $MgSO_4$. Add components just before use, using sterile technique; do not autoclave.]

IP buffer [15mM HEPES pH 7.6, 10mM KCl, 1.5mM $MgCl_2$, 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 case

No Contamination found

In case of no contamination the worms will be seperated 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

step case

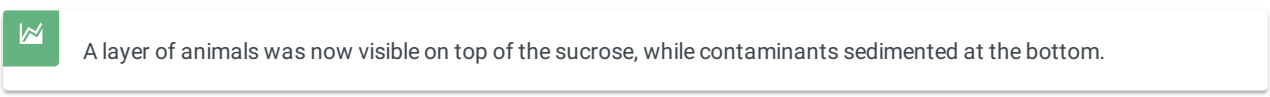
Contamination found

In this case, clumps of bacteria or fungal balls of hyphae are seen in the culture. Depending on the application, the bacteria can be removed and C.elegans used for experiments or the culture has to be discarded in case of sensitive experiments

10 After the second wash step, animals were resuspended in 20 ml of ice-cold M9 lacking $MgSO_4$, followed by the addition of 20 ml of ice-cold 60 % sucrose in H_2O.

11 After vigorous mixing of the sucrose/worm mixture, 4 ml of ice-cold M9 lacking $MgSO_4$ was gently layered on top, and the worms were centrifuged at 400 g for 2 min at 4 °C.

The sucrose float steps were performed as quickly as possible, as otherwise, the layer of animals failed to form properly.



A layer of animals was now visible on top of the sucrose, while contaminants sedimented at the bottom.

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