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# Setting up a liquid culture and harvesting C.elegans

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

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## Materials

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 2 steps

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