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Harvesting OP50 for C. elegans liquid culture

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Protocol status: In development
We are still developing and optimizing this protocol

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Troubleshooting

Harvesting OP50

1 After 8 hr incubation, use 2 ml of E. coli OP50 LB culture to inoculate each of 3 × 250 ml LB. Place flasks in shaking incubator (180 rpm) O/N. 17:00:00

37 °C

2 Weigh 3 × 50 ml centrifuge tubes and write weight of the empty tube on the tube.

3

Day 2. Using a serological pipette, aliquot 40 ml of O/N E. coli OP50 culture into the pre weighed centrifuge tubes. Centrifuge in swing bucket centrifuge at 3,220 x g, 10°C, 10 min. 00:10:00

10 °C

4

Carefully decant the supernatant, repeat step 4 till all the culture is used up. Keep the tube inverted with lid on and leave to stand for several minutes. Using a pipette remove any excess supernatant that may have collected in the lid. Weigh the tube and calculate the weight of the pellet. go to step #4

5 Calculate the volume needed to provide a suspension of 30 g/L and mark this volume on the tube. Date, label and place tubes at -20 °C for use within 1-3 months or at - 80 °C if storing for longer than 3 months.

6 Prepare bacterial suspension for growing nematodes; allow bacterial pellet to thaw out and add required volume of S complete medium to each tube, vortex gently to resuspend pellet, pool contents of different tubes to obtain the volume required. Work under sterile conditions.