

May 18, 2023

Transferring *C. elegans* to S-basal to grow them in liquid culture

DOI

<https://dx.doi.org/10.17504/protocols.io.81wgbyj41vpk/v1>

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Protocol Citation: Muhammad Zaka Asif, Man Shah, Yosef Smadi 2023. Transferring *C. elegans* to S-basal to grow them in liquid culture. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.81wgbyj41vpk/v1>

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Protocol status: Working

We use this protocol and it's working

Created: May 18, 2023



Last Modified: May 18, 2023

Protocol Integer ID: 82033

Keywords: worms in liquid culture, liquid culture this protocol, liquid culture, synchronized worm, worm, production, production of natural product, hp derivative

Abstract

This protocol describes day 2 of our workflow to grow worms in liquid culture to induce the production of natural products (in this case, 1-HP derivatives). In this protocol, we get an approximate count for the number of life-stage synchronized worms we have, ensure to concentrate them to 30,000 worms/ml, and provide *E.coli* OP50 for them to be able to progress



- 1 Remove flasks with L1-arrested worms from the shaker
- 2 Swirl vigorously for at least 30 seconds, then collect a 1 uL aliquot and place onto a glass slide for inspection. Use a 0.5 uL aliquot if there are more than 100 worms in the 1 uL aliquot.
- 3 Count using a tally counter. Repeat for at least 3 trials (may need to do more if you don't shake enough initially and end up empty).
- 4 Once all the aliquots are collected this way, pour each of the flasks volume into a 15 mL flip top tube. Measure the volume. Determine the total population from these two data, as shown below.
- 5
$$\text{Total population} = (\text{total volume in mL}) \times (\text{concentration of worms/uL}) \times 1000 \text{ uL/mL}$$
- 6 Centrifuge the flip top tubes at 1200 rpm for 3 minutes.
- 7 Aspirate out supernatant, leaving about 0.5 mL behind.
- 8 Dilute in enough S-basal to achieve a concentration of 30,000 worms/mL.
- 9 The minimum volume should be 0.5 mL if there are less than 5,000 worms total.
- 10 Shake vigorously to ensure worms are transferred to solution. Pour into the same flask as before (to minimize transfer loss).
- 11 Add enough bacteria for a concentration of 2% E. Coli. Make sure the final worm concentration is 30,000 worms/mL (essentially, add less S-basal based on how much bacteria is being added).
- 12 Repeat steps 1-10 for controls with worms (one for DMSO and one for bacteria only).



- 13 If using negative controls, repeat steps 9 and 10 (no worms).
- 14 Essentially, as negative controls, the flasks will have the same total volume of solution (identical S-basal and bacteria) as the earlier flask. They will be exposed to the same conditions just without any worms. The negative controls will later be separated into bacteria-only, bacteria + DMSO, and S-basal + 1-HP in Day 4.
- 15 Put the flask back in the shaker