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Symbiotic Dose-50 (SD50) for Vibrio fischeri strain to colonize Euprymna scolopes V.1

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ABSTRACT

This protocol details symbiotic dose-50 (SD_{50}) for *Vibrio fischeri* strain to colonize *Euprymna scolopes*.

ATTACHMENTS

680-1440.docx

MATERIALS

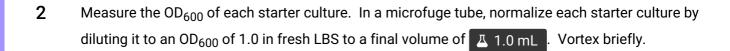
Materials needed:

- 1. Culture tubes
- 2. LBS medium
- 3. Shaking incubator
- 4. Spectrophotometer
- 5. Tumblers
- 6. Freshly hatched E. scolopes squid
- Filter-sterilized seawater (FSSW): Instant Ocean (Spectrum Brands, Blacksburg, VA) mixed according to instructions provided by manufacturer. Filter through 0.22 surfactant-free filter.
- 8. Microfuge tubes
- 9. 50-mL conical tubes
- 10. LBS plates
- 11. Vials
- 12. Luminometer

Keywords: Preparation of V. fischeri Cultures, Preparation of Juvenile E. scolopes, Inoculation Phase, Measurement of Bioluminescence, Euthanasia and Storage of Animals, Scoring of Bioluminescence

Preparation of *V. fischeri* Cultures





Initiate an intermediate culture by inoculating \square 3 mL LBS in a fresh culture tube with \square 30 μ L of the normalized cell suspension. Incubate at \square 28 °C shaking at \square 200 rpm

Selection and Preparation of Juvenile *E. scolopes*

- 5 For each group, add 👲 50 mL FSSW to a tumbler.



6 Transfer animals individually to each tumbler.

3

Note

To minimize bias, add an animal to the tumbler of a different group with each transfer.

Preparation of Inoculums

- For each strain, when the turbidity of culture is $OD_{600} = 0.8-1.0$, transfer culture volume equivalent to \square 1 mL of $OD_{600} = 1.0$ to a microfuge tube.
- **8** Concentrate cells by centrifugation.



- 8.1 Concentrate cells by centrifugation at supernatant, add 4 0.9 mL FSSW, and resuspend the pellet. (1/2)
- Concentrate cells by centrifugation at 0.9 mL FSSW, and resuspend the pellet. (1/2)
- Prepare a serial dilution by transferring \square 100 μ L of the cell suspension described in Step 8 into \square 0.9 mL FSSW in a microfuge tube (10-1 dilution). Then, continue ten-fold dilutions until the desired dilution range has been achieved.

Note

Note that three-fold dilutions can be used instead for greater resolution.

Prepare a control for an apo-symbiotic group by transferring I 1 mL FSSW to a microfuge tube.

2m 30s

11

conical tube containing <u>I</u> 50 mL FSSW and invert several times to mix.

For each group, transfer 🚨 100 µL from the corresponding microfuge tube into a 50-mL



Inoculation Phase

12 To initiate the inoculation phase, pour the cell suspension into the corresponding tumbler to bring the total volume to A 100 mL. Repeat for the control described in Step 10.

13 Sample tumblers by plating A 100 µL onto solid LBS medium in triplicate and incubate the Overnight plates at 1°28 °C



Note

Note that for high inoculum levels, a dilution may be necessary to obtain countable CFUs. For low inoculum levels, it may be preferable to use the known dilution factor from more concentrated inoculums to estimate the corresponding abundance of V. fischeri.

14 After 3.5 hours, wash the animals by serially transferring them as a group into a tumbler containing <u>I</u> 100 mL FSSW twice, with <u>(*)</u> 00:05:00 between transfers.

- 15 Transfer animals into vials containing A 4 mL FSSW, with one animal per vial.
- 16 Store animals in a room that has a 12-h day/12-h night light cycle.

Measurement of Bioluminescence

- 17 After 16-18 h, transfer animals to clean vials containing 4 mL FSSW.
- 18 Using a luminometer, measure the luminescence emitted by each sample.

Euthanasia and Storage of Animals

- To initiate the anesthesia step, transfer each animal with seawater (total volume of ___ 0.5 mL) to a microfuge tube and place ___ On ice .
- After 00:05:00 , add 0.5 mL cold 6% ethanol/FSSW to each microfuge tube and keep 0 on ice .
- After 00:15:00, remove the liquid volume from the tube and store the anesthetized animal at 8-80 °C, thereby completing euthanasia.

Scoring of Bioluminescence

- Use the luminescence measurements of the apo-symbiotic group to determine the 99.9th percentile, above which animals are considered to be bioluminescent.
- Score each animal as symbiotic or non-symbiotic by comparing the corresponding luminescence measurement with the bioluminescence cutoff defined in Step 22.

Determining Inoculum Levels

5m

- Count CFU on the inoculum plates generated in Step 13. Also verify that no CFU are present on the apo-symbiotic control plates.
- Calculate the concentration of CFUs in each inoculum cell suspension described in Step 9 by dividing the CFU counts by the volume plated (in mL) and multiplying by the dilution factor, if any.

Calculation of SD₅₀

- For each strain, generate a table with the number of symbiotic and non-symbiotic animals at each inoculum concentration, with rows arranged in order of highest to lowest concentration.
- 27 Prepare two additional columns containing adjusted counts for
 - 1. animals that could be assumed to be symbiotic at higher inoculums and
 - 2. animals that could be assumed to be non-symbiotic at lower inoculums.
- Calculate the adjusted percent of symbiotic animals at each inoculum by dividing the adjusted counts of symbiotic animals by the total adjusted animal counts in the corresponding row.
- 29 Calculate the SD₅₀ using the equation:

$$SD_{50} = 10^{\log(DF^X)} + \log(c)$$
, where

- X = [(50%-a)/(b-a)] and
- a = the adjusted percent symbiotic below 50% closest to 50%.
- b = the adjusted percent symbiotic above 50% closest to 50%.
- c = the inoculum concentration of the adjusted percent colonized below 50% closest to 50%.
- DF = the dilution factor or fold-change difference between groups in the experiment.