Hydra Dissociation/Reaggregation

Yashodara Abeykoon¹, Adrienne Cho¹, Celina Juliano¹

¹UC Davis

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Yashodara Abeykoon

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION


ATTACHMENTS

Dissociation Medium
Recipe.pdf

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Guidelines

Note: This protocol was adapted in the Juliano laboratory from published protocols and can tolerate a lot of variation.

- [ST1] This step removes the tentacles, which will not participate in development of an aggregate.

- [ST2] Aim for reducing the Hydra pellet volume by ~1/2 (i.e. shearing approximately half of the available tissue.) Over dissociation can damage the cells, resulting in a reduction in aggregate survival/development.

- [ST3] Depending on the cell density, 50-200 ul aliquots are generally sufficient in size to develop into one or more Hydra. Aggregate size will dictate the number of heads formed. Larger aggregates are more likely to survive, up to a point. Ultimately, you have to play with this step to determine what works best for you and your experiment.

- [ST4] We have had success with reduced times and speeds.

- [ST5] Can alternatively gently pipette to dissociate pellet from the tube wall, but the passive method minimizes further disruptions to the pellet.

- [ST6] The 50/50 mix of DM/HM and 100% DM conditions resulted in similar rates of survival, but it seemed that the 50/50 mix may slightly increase the rate of development. Immediate transfer to 100% HM results in low survival rates.

- [ST7] In our hands, aggregates are healthier if provided with antibiotics.

Materials text

- Hydra medium (HM) with and without Penicillin/streptomycin (P/S)

Hydra medium is made from two stock solutions:

**Stock Solution 1 (1000X)**

42.18 g CaCl₂ x 2H₂O
Up to 1 L Milli-Q Water
Filter Sterilize

**Stock Solution 2 (100X)**

8.116 g MgSO₄ X 7H₂O
4.238 g NaHCO₃
1.0985 g K₂CO₃
Up to 1 L Milli-Q Water
Filter Sterilize

**20 L Hydra Medium**
20 mL of Stock solution 1
200 mL of Stock solution 2
Up to 20 L with Milli-Q Water
Stir on stir plate for 45 min

- Dissociation medium (DM), filtered, chilled to 4°C, pH to 6.9-7

Please refer to attached recipe in "Abstract".

**NOTE: We used HEPES instead of TES**

- Glass Pasteur pipette
- Eppendorf tubes
- 24-well plate
BEFORE STARTING
Refer to guidelines when [ST#] is mentioned in the steps

### Dissociation of Hydra

1. Wash 30 Hydra several times in fresh Hydra Medium (HM) to remove debris

2. Transfer Hydra to 1.5mL Eppendorf, remove media, and add 1mL 4°C Dissociation Medium (DM)

3. Incubate Hydra at 4°C for 30-60 min (can incubate several hours without obvious detriment/benefit)

4. Resuspend Hydra in 1mL fresh 4°C DM

5. Shear by glass Pasteur pipette ~10-15x until supernatant becomes slightly turbid, allow Hydra to settle, and discard supernatant [ST1 - refer to guidelines]

6. Add 1mL fresh 4°C DM and shear by pipetting up and down with glass pasteur pipette ~1-1.5 min until solution becomes turbid, and approximately half of the available tissue is dissociated [ST2 - refer to guidelines]

7. Let remaining large pieces settle. The supernatant contains the cells that will be collected and used for reaggregation

### Generating aggregates capable of developing into whole animals:

8. Aliquot supernatant (dissociated cell suspension from step 7) to new Eppendorf tubes at an approximate desired cell density [ST3 - refer to guidelines]

9. Centrifuge aliquots in microcentrifuge at 800g for 6 min [ST4 - refer to guidelines]

10. Invert tubes for ~5-10 min to allow pellet to passively separate from the tube wall [ST5 - refer to guidelines]

11. Transfer pellet to a 24-well plate containing a 50/50 mixture of DM and HM+Penicillin/Streptomycin (P/S) or 100% DM [ST6 - refer to guidelines]

12. Periodically during development, replace the medium with HM+P/S
If starting from 50/50 DM/HM+P/S, depending on the duration of the experiment, make the first medium exchange by 12hpa or 24hpa, and make subsequent exchanges at ~24h intervals for the duration of the experiment.

If starting with 100% DM, exchange into 50/50 DM/HM+P/S at ~4 hours post aggregation (hpa), exhange into HM+P/S 100% at ~16hpa, and then continue to exchange medium every ~24 hours [ST7 - refer to guidelines]

Enjoy your shiny, new *Hydra*