Hydra Dissociation/Reaggregation

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1 *Works for me* dx.doi.org/10.17504/protocols.io.6tyhepw

Open Hydra

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Flick KM, Bode HR (1983). 'Dissociating Tissues into Cells and the Development of Hydra from Aggregated Cells' in Lenhoff, HM. *Hydra: Research Methods*. New York: Plenum Press, pp 252-259.

Dissociation Medium Recipe.pdf

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 particpate 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 resuls 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 $CaCl_2$ x 2H2O
Up to 1 L Milli-Q Water
Filter Sterilize

Stock Solution 2 (100X)

8.116 g $MgSO_4 X 7H_2O$
4.238 g $NaHCO_3$
1.0985 g K_2CO_3

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

13 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.

14 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 exange medium every ~24 hours **[ST7 - refer to guidelines]**

15 Enjoy your shiny, new *Hydra*