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Hydra Dissociation/Reaggregation

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Protocol status: Working

We use this protocol and it's working

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Protocol Integer ID: 27224



Attachments



Dissociation Medium ...

488KB

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 $\sim 1/2$ (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 μL 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

- 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 $\text{CaCl}_2 \times 2\text{H}_2\text{O}$

Up to 1 L Milli-Q Water

Filter Sterilize

Stock Solution 2 (100X)

8.116 g $\text{MgSO}_4 \times 7\text{H}_2\text{O}$

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 start

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), exchange into HM+P/S 100% at ~16hpa, and then continue to exchange medium every ~24 hours **[ST7 - refer to guidelines]**
- 15 Enjoy your shiny, new *Hydra*