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🌐 Cold-shock protocol to bleach Aiptasia

📁 In 1 collection

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Aiptasia Symbiodiniaceae Model System

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ABSTRACT

This protocol describes how to use a short-term cold shock to render Aiptasia aposymbiotic. After the cold shock it includes two possibilities to proceed one faster one with usage of DCMU and a slower and more gentle one with only keeping the animals in the dark.

GUIDELINES

Preparing DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) stock solution:

0.0313 M DCMU in 95-100% ethanol.
 For 50 ml, dissolve 7.3 mg in 50 ml ethanol.

For an end concentration of 50 μ M add 32 μ l of the stock to 200 ml of artificial seawater.

MATERIALS

MATERIALS

- ⊗ DCMU Sigma – Aldrich Catalog #D2425
- ⊗ Artificial seawater Contributed by users
- ⊗ Fluorescence stereomicroscope Contributed by users

SAFETY WARNINGS

DCMU is toxic and suspected to be carcinogenic.

[https://chemicalsafety.com/sds1/sdsviewer.php?id=30234487&name=3-\(3,4-Dichlorophenyl\)-1-methylurea](https://chemicalsafety.com/sds1/sdsviewer.php?id=30234487&name=3-(3,4-Dichlorophenyl)-1-methylurea)

BEFORE START INSTRUCTIONS

Fluorescence filters:

Every wavelength between 400 and 550 nm efficiently excites the algal chlorophyll. The chlorophyll fluorescence emission peaks between 650 and 720 nm.

This means that most RFP filter sets with a wide bandpass or longpass emission filter at above 600 nm will work well.

GFP longpass filter sets will work as well but only in combination with an RGB camera to distinguish red algal fluorescence and green autofluorescence of the anemones themselves.

These numbers are only rough estimations based on a few algal strains. For purchasing new filter sets it will be still best to test them before buying.

Preparation

- 1 Prepare a tank of anemones.**
It is best (but not critical) if you let them sit in the tank until they stick to the sides/bottom of the tank.
- 2 Chill a large volume of seawater to 4°C.**

Initial treatment

- 3 Pour out the seawater in the Aiptasia tank and replace it with the 4°C seawater. Place the entire tank into a 4°C refrigerator (or cold room).**
- 4 Incubate at 4°C for 4 hours.**

04:00:00 Cold-shock

- 5 **Remove the tank from 4°C, pour out the cold water, and replace with room-temperature seawater.**
The animals will be spitting out boluses of algae, which you will see as dark brown balls next to the anemones
- 6 **Repeat the water change 2-3 times by pouring out the tank water and replacing it with room-temperature seawater.**
- 7 **Place the tank into darkness (we use dark cabinets) at room temperature for 1 – 2 days. Continue to replace seawater with room-temperature seawater once a day.**

Complete bleaching

- 8 ***You now have two options: a faster method or a slower method. The faster method works quickly but is harsher on the anemones; the slower method is more gentle on the anemones but takes longer.***
Step 8 includes a Step case.
Fast and harsh bleaching
Slower and more gentle bleaching

Fast bleaching

step case

Fast and harsh bleaching

Using DCMU to remove algae faster but with more stress to the animals

- 9 **Prepare seawater containing DCMU (or 'diuron': 3-[3,4-dichlorophenyl]-1,1-dimethylurea). We generally use a final DCMU concentration in seawater between 10 µM and 50 µM.**
Higher concentrations work faster, but are harsher on the anemones so you may need more frequent water changes.
- 10 **Remove the tank from the dark cabinet. Replace tank seawater with the DCMU-seawater. Incubate at room temperature in constant light for several weeks.**

- 11** Replace the water with DCMU-seawater once or twice a week (depending on how the anemones look: when they appear very unhappy, replace the water). Feed anemones with brine shrimp at least once a week; more feeding is better, so feed 2 – 3 times a week if possible.
Importantly, after each feeding the water must be changed the next day.
- 12** You can start screening the anemones under a fluorescence stereoscope after 2 – 3 weeks to check for the presence of algae. Continue incubating with DCMU until anemones appear aposymbiotic.
See guidelines section for information about fluorescence filters.
- 13** When anemones appear aposymbiotic, incubate first a few of them in room-temperature seawater in the light (without DCMU) for 1 week and then check for algal presence under a fluorescence stereoscope.
This step ensures there are no algae still in the anemones: if any are present, they will populate quickly over the week and become detectable.
If they still contain algae, continue the DCMU treatment. If they remain free of algae, they are fully aposymbiotic.