Preparing small, live Aiptasia polyps for confocal microscopy

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Abstract

This protocol, which was developed in 2006 by Santiago Perez while in the Weis lab, provides a way to temporarily immobilize small Aiptasia for live imaging on a confocal microscope. As written, the protocol is used for inverted confocal microscopes, but the glass-bottom dishes can be replaced with a normal glass slide and coverslip for an upright microscope.

Citation: OSU Weis Lab,Trevor Tivey Preparing small, live Aiptasia polyps for confocal microscopy. **protocols.io** dx.doi.org/10.17504/protocols.io.rigd4bw
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Guidelines

NOTE: If you are going to take images that don't require long scanning times (which would thus require well-immobilized anemones) skip embedding in agar and simply use the $MgCl_2$/seawater medium.

Protocol

Step 1.

Make a 1:1 solution of regular seawater (filtered/sterile optional) and 0.37M $MgCl_2$ (relaxing solution). Place anemones in the solution and within 10 minutes they will relax (i.e., start expanding and become unresponsive to touch).

NOTES

This can be done directly on a MatTek glass-bottom culture dish for inverted microscopes.

Step 2.

Add any fluorescent, vital dyes to the sample during the relaxation step. Typical load times for vital dyes is 30-60 minutes.

Step 3.

After the animals are relaxed, remove relaxing solution and add a few drops of new relaxing solution + 0.5-1.5% low melting agarose, boiled then allowed to cool to 28-30°C (right before becomes a gel). Let cool and add liquid media, filling up most of the glass-bottom dish (3mL).

NOTES

If you are not going to embed in agarose, add just enough relaxing solution to cover the anemones over the shallow well created by the coverslip circle. Watch out for drying out though!!

Step 4.

Adults can be recoverd from the agarose after imaging. Remove the the agarose surrounding the anemones, and place the anemones back into seawater.