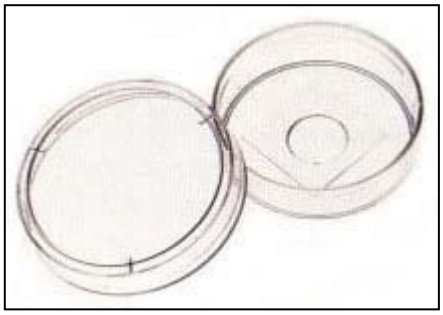
Preparing live Aiptasia tinies for confocal microscopy
Prepared by Santiago Perez; 3/22/06

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 MgCl2/seawater medium.

- **Inmobilizing anemones:**
  A magnesium chloride solution works great! Make a 1:1 solution of regular seawater (filtered/sterile optional) and 0.37M MgCl2. Place anemones in the solution and within 10 minutes or less they start expanding and become unresponsibe to touch.
- This can be done directly on a MatTek glass-bottom culture dish (http://www.glass-bottom-dishes.com/index.html; see picture)



- In addition, I typically add fluorescent dyes at this stage (i.e. I make the dye mix into the relaxing solution).
- Most dyes take 30-60 minutes to load.
- Once expanded, remove relaxing solution and add a few drops of the same solution + 0.5-1.5% low melting agarose, boiled then allowed to cool to 28-30C (right before becomes a gel).
- Let cool and add liquid media (about 3mls in the above dishes)
- These dishes fit into a temperature controlled stage of the Zeis confocal microscope. Talk to the confocal trainers on use of the device (this is required by CGRB).
- If you are not going to embed in agarose, add just enough MgCl solution to cover the anemones over the shallow well created by the coverslip circle. Watch out for drying out though!!