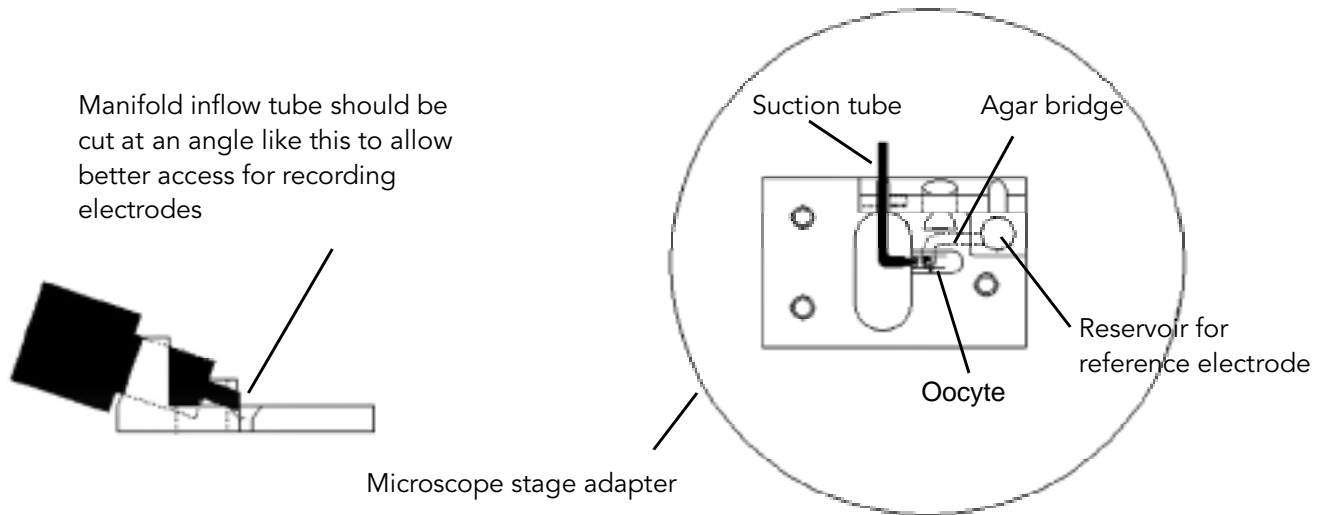


Oocyte Perfusion Chamber (OPC-1) Instructions

Fitting the manifold into the chamber

In order for inflow tubing not to obstruct the movements of recording electrodes, the tubing should be cut so that it does not occupy any space in the working volume of the chamber. The outer surface of inflow tubing can be covered with Vaseline to prevent solution diffusion between tubing and the chamber.



1. Prepare agar solution to fill the bridge between the well (where an oocyte will be positioned later - see diagram) and the reference solution reservoir. This is easily accomplished with the help of a syringe previously filled with melted agar solution. Use a short piece of silicon tubing (instead of a needle) that fits into the reference reservoir. Fill the bridge by squeezing the agar out of the syringe. Remove excess agar from the well with forceps or a needle. Dig out the well completely. Only leave a tiny amount of agar if lower working volume is desired. If the oocyte is too high, it may float out of the well during perfusion.
2. Fill the reference reservoir with a 3 M KCl solution and put a reference electrode inside the reservoir. To keep the electrode in place, you might put the electrode inside silicone tubing, which could be squeezed into the slot above the well.
3. Fill your perfusion system with solutions, and insert the manifold in the chamber. Briefly open one of the valves and fill the chamber with a solution. Remove any air bubble from the chamber.
4. Pick up an oocyte with a Pasteur pipette (or a plastic pipette) and gently squeeze it into the well. You can **temporarily turn outflow suction off** to prevent the oocyte from being sucked into the vacuum trap.
5. Penetrate the oocyte with electrodes.

Cleaning the chamber

After you are finished with experiments, it is a good idea to remove the agar solution from the chamber. Remove the three screws that fasten the chamber's body and the base (microscope stage adapter). Remove the agar from the bridge with any thin instrument (needle). Clean the chamber with warm distilled water, and let it dry before screwing it together again.

Tips on usage

1. If you are using a bath clamp with two reference electrodes, insert them both inside the reference reservoir.
2. If you find filling the agar bridge with an agar solution too time consuming, you can mount both electrodes inside the well where the oocyte will be placed. The electrodes can be wired through the opening for the agar bridge. Fill the bridge with melted wax to prevent solution leakage. One reference electrode can be bent along the wall of the working volume. The other can be placed inside the inflow tubing. Be careful, the two electrodes must not touch. After you have positioned the electrodes inside the chamber, apply some Clorox bleach to cover the electrodes with AgCl.

