Oocyte Perfusion Chambers
OPC3, OPC4

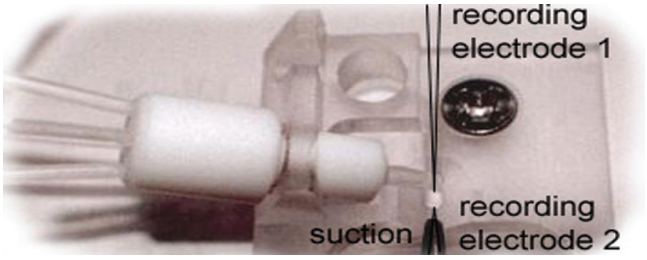
1. Remove the chamber insert from the stage plate. The insert can be easily removed after relaxing and swinging out three flat springs. Put a thin layer of Vaseline or mineral oil on the bottom of the insert. Put the insert back inside the stage adapter and fix it with springs. If using the chamber with glass bottom, put the glass slip first into square cutout on the bottom of the stage plate. Putting little oil or Vaseline on the edges of the groove ensures leak-proof assembly and prevents liquid spill on optics bellow the chamber. Note: It is recommended to wash the chamber from possible debris before the first use.

2. Prepare agar solution and fill the bridge between the well (where an oocyte would be positioned) and a reference solution reservoir. The agar can be easily put in the bridge with a help of a syringe previously filled with hot 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 of agar from the well with forceps or a needle.

3. 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 of silicone tubing, which can be squeezed into the opening near and above the reservoir.

4. Fill perfusion system with solutions. Put the manifold in the chamber. Briefly open one of the perfusion valves and fill the chamber with a solution. Remove air bubbles from the chamber if any.

5. Pick up an oocyte with a Pasteur pipette (or a plastic pipette) and gently squeeze it into the well. You can temporarily lift the outflow tubing to raise the solution's level and to prevent oocyte from sucking into the vacuum trap, or just turn outflow temporarily off.



6. Penetrate the oocyte with electrodes. The healthy oocyte, penetrated with two electrodes and slightly adjacent to the little step near the suction tubing, will stay in place after the solution flow is turned on. Adjust the height of the suction tubing to a desired solution level in the well. The higher the suction tubing the larger the solution volume in the well and the slower the solution exchange rate.

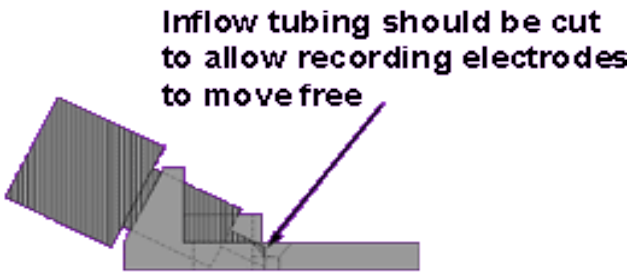
You are ready to use your the perfusion chamber now.

Cleaning the Chamber

In order to remove agar solution from the chamber, remove three screws that fasten the chamber's body to the base (stage plate). Take the agar out of the bridge with any thin instrument (needle). Clean the chamber with warm distilled water and let it dry before screwing it together again.

Fitting the Manifold Into the Chamber

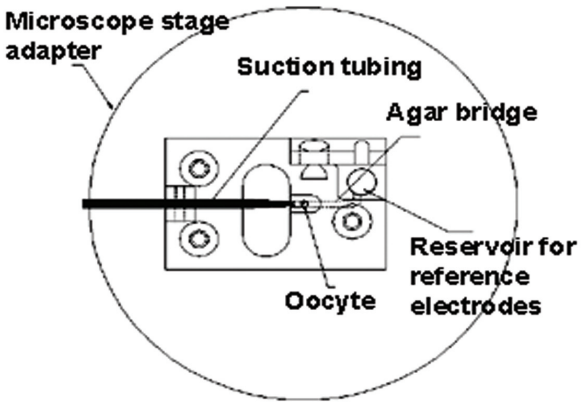
In order for inflow tubing not to obstruct the movements of recording electrodes, the inflow tubing should be cut so that it does not occupy any space in the working volume of the chamber. The outer surface of inflow tubing can be covered with Vaseline to prevent solution diffusion between tubing and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



Note: Due to continuous improvements the actual design might differ.

Usage Tips

1) If you are using a bath clamp with two reference electrodes, put both electrodes inside the reference reservoir.



2) You might find filling the agar bridge with an agar solution and keeping the reference reservoir filled with KCL solution a time consuming process. In this case you can put both reference electrodes inside the well where oocyte will be positioned during the experiment. The electrodes can be wired through the opening for the agar bridge. Afterwards, fill the bridge with melted wax to prevent solution licking out. One reference electrode can be bent along the wall of the well, which will be filled with solution during experiment, or just touch the solution in the well. Another, if you use two reference electrodes, can be put inside inflow tubing. Be careful, two electrodes must not touch.) You do not have to put long electrodes into the well, but use only short parts (1-2mm) of chlorinated electrodes sticking out of the bridge (this will save trouble of arranging the reference electrodes inside the well.) To cover the electrodes with AgCl, put a few droplets of Clorox into the chamber. Do not forget to wash Clorox out of the chamber with distilled water before the experiment.