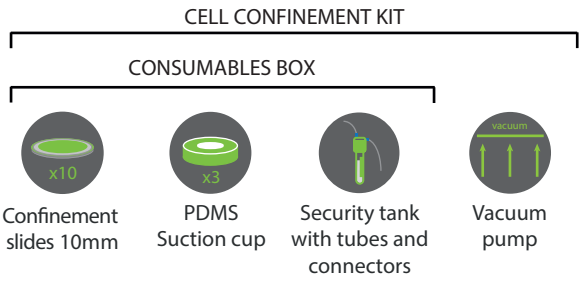
CELL CONFINEMENT KIT

Material included



Recommended material

Ethanol 70%
Delicate wipes
Tweezers
Adhesive tape

A. PREPARING THE SUCTION CUP

1. At least 1 h before the experiment, incubate the confinement slide in the cell culture medium that will be used for the experiment to equilibrate the PDMS with the medium. The structured PDMS side of the slide should face upward. (1)
2. Before the experiment, clean the cell confiner with 70% ethanol, and dry it carefully with absorbing paper. (2)
3. Clean the bottom face of the device with adhesive tape to remove dust particles.
4. Plug the metal part of the suction cup to the tube and the tube to the controllable vacuum source. After the device is well sealed, the membrane sustaining the piston should be slightly deformed.
5. Tune the controller to 3 kPa (30 mbar)(3). At this pressure, and with a good seal, the device sticks to the substrate, but the confinement slide does not yet touch the cells.

B. PREPARING THE CONFINEMENT SLIDE

6. Prepare an absorbing paper to dry the back side of the confinement slide.
7. Pick up the confinement slide with tweezers, and dry its back side on the absorbing paper by placing it onto the paper. (4)
8. Place the confinement slide onto the piston of the cell confiner, structured side up. The slide should stick naturally onto the piston.

C. CONFINING THE CELLS

9. Put the device in contact with the cell culture substrate. (5) At this point, cells can be observed before confinement. (6)
10. To confine cells, slowly decrease the pressure down to 10 kPa (100 mbar). By observing the cells during this time, one should see the confinement slide touching cells at approximately 5 kPa.
11. Confinement can be released if the pressure is increased back to 3 kPa.
12. After switching off the pump, you can clean the suction cup and the confinement slide with water and store them in a dry place. For future use, they can be sterilized with ethanol.

(1) Be careful not to loose the orientation of the slide during manipulation; it can be difficult to recover.
(2) Be careful not to leave the device in contact with the ethanol for a long time, or it will absorb the ethanol and become toxic to cells.
(3) Please be aware that you can only read the vacuum/pressure value if you have the AF1 controller.
(4) If the back side is not totally dry, it will not stick onto the device and can be lost during handling.
(5) Make sure not to deform the device to achieve a good seal of the device cavity. Also, be careful not to push on the center of the device, or cells could be crushed by the device.
(6) The petri dish will not move during the confinement operation, and several cells can be observed simultaneously.

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