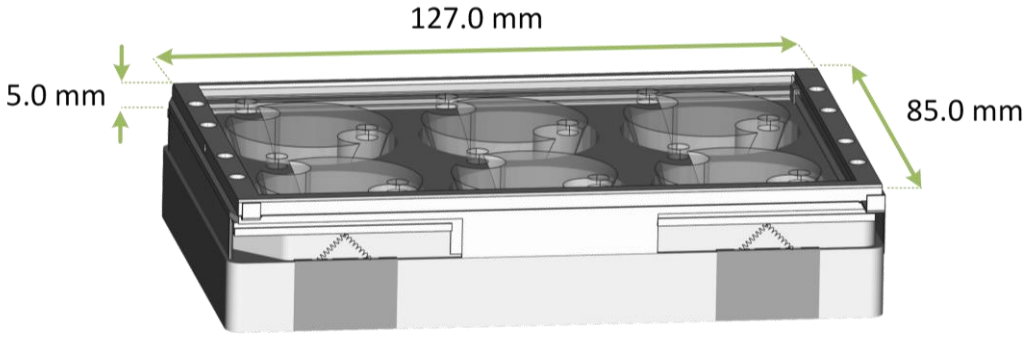
Material Included:

4DCell CSOW 620
Confinement coverslip (12 unites)
PDMS pistons (12 unites)
Scalpel
Tape
Caps

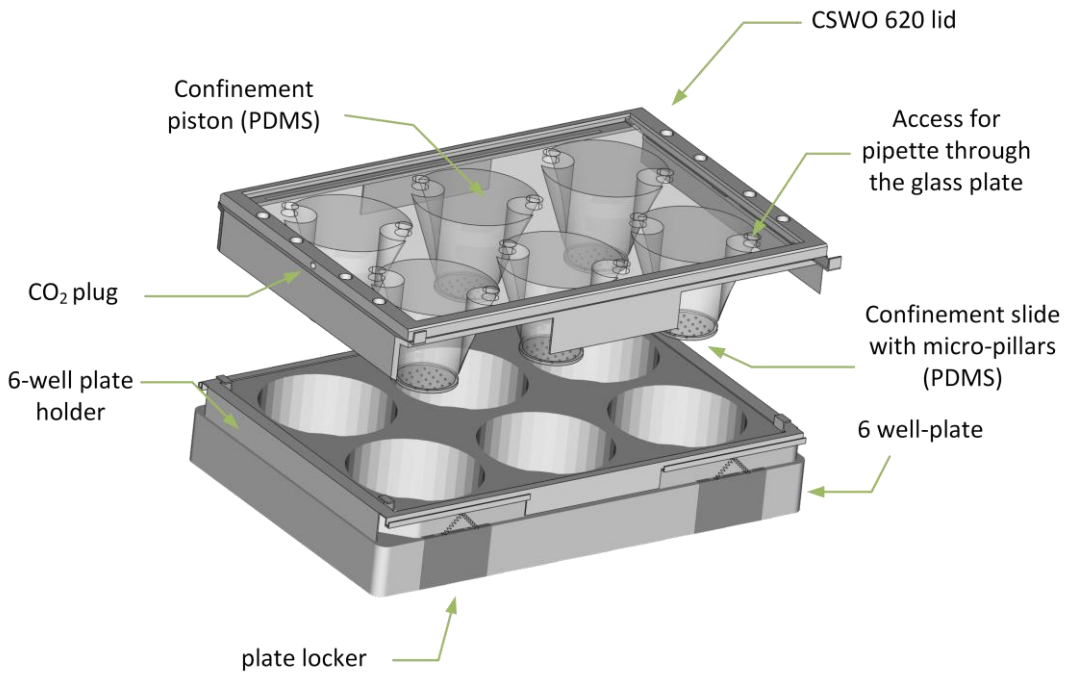
Other recommended material:

Ethanol 70%
Tweezers
6-well plates (Glass Bottom Mattek)

CSOW 620 dimensions:

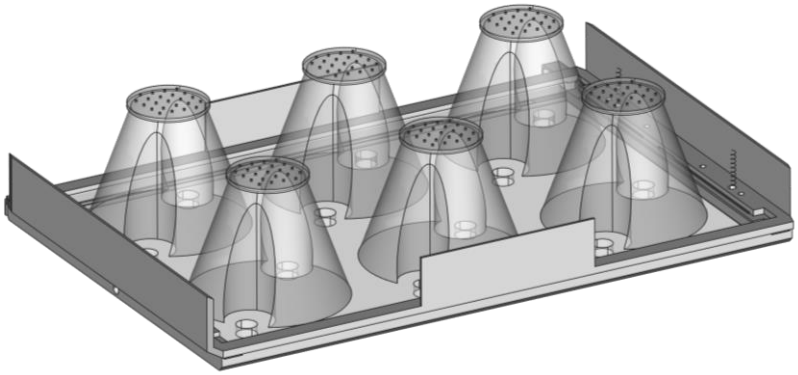


CSOW 620 parts:



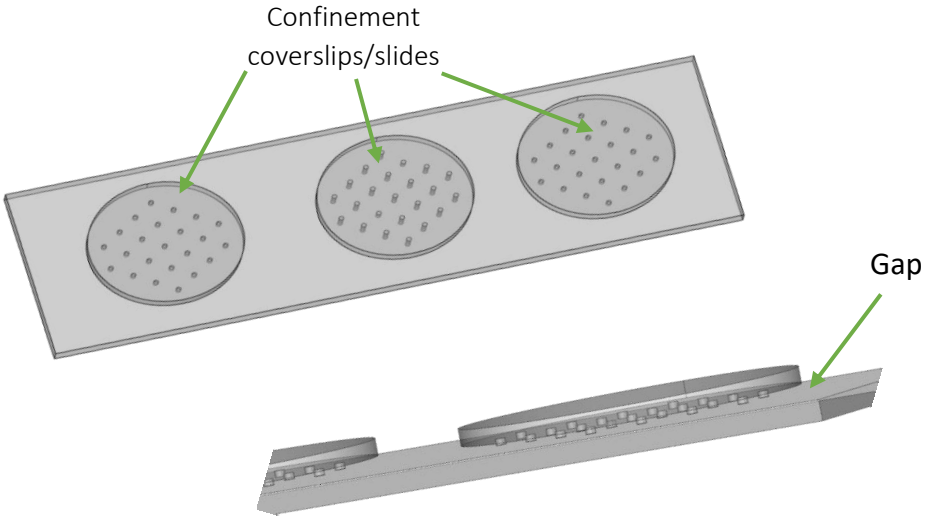
Handling:

1. Clean the two parts of the CSOW 620 confiner, the lid (top part) and holder (bottom part), using absorbing paper and 70 % ethanol.
2. Clean the pistons using ethanol 70%. If the PDMS pistons have particles of dust these can preclude the good attachment to the glass. In this case, the tape provided in the kit can be used to remove the particles previously to cleaning them with ethanol 70%.
3. Attach the pistons to the CSOW 620 lid. Make sure the indentations of the pistons are aligned with the holes in the glass plate.



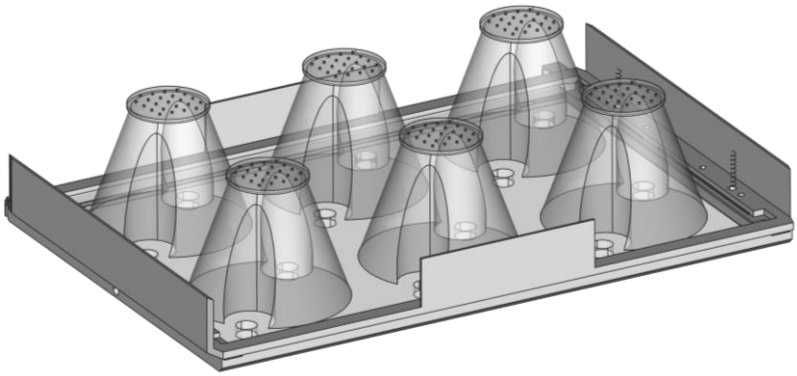
The PDMS is usually sticky, enabling it to be attached to glass very easily. The holes can be used to pipette media, aspirate it, etc. The holes can be closed with the caps provided in the kit. If you find difficult to remove the caps, please use a tweezer to do so.

4. Remove one confinement slide from the microscope slide where they were shipped. The pillars are facing down. You can use a blade to gently remove it. Place the blade in the small gap between the confinement slide and the microscope glass slide. Afterwards, tilt the blade so that the confinement coverslip detaches from the microscope glass slide.



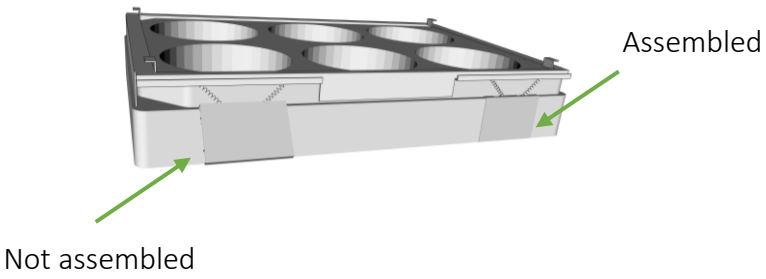
The confinement coverslips must be cleaned with ethanol 70% before being used. Clean the back of the coverslips with absorbing paper before placing them on the top of the pistons.

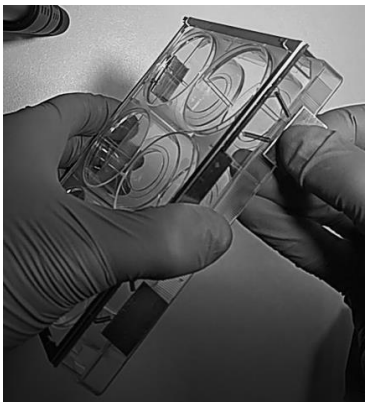
5. Place the confinement slide on the PDMS piston with the pillars facing up as depicted in the figure bellow:



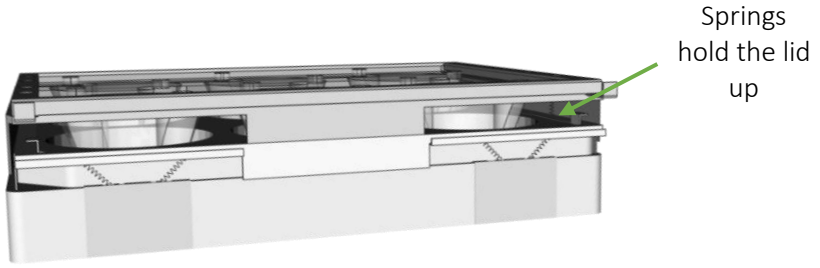
Incubate the pillars and the confinement slides in culture medium to equilibrate the PDMS at least during one hour. To do this you can put the lid on the top of a 6-well plate. The springs attached to the inside part of the lid will preclude the confinement slides to touch the bottom of the plate. Note that PDMS absorbs small hydrophobic molecules from the medium. Therefore, if drugs are used in the experiment, these drugs should be present during this incubation step.

6. To assemble the bottom part to the 6-well plate, gently pull the pads down to grab on the bottom part of the plate.

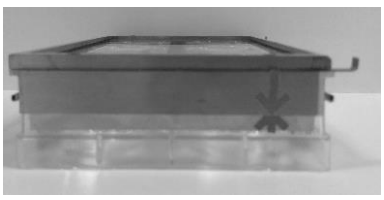




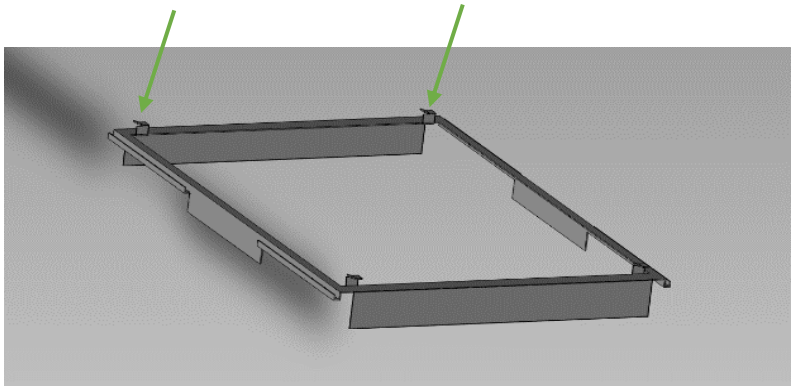
7. Place the lid on the top of the assembled holder part. Allow it to be in the resting position, where the springs hold the lid up.



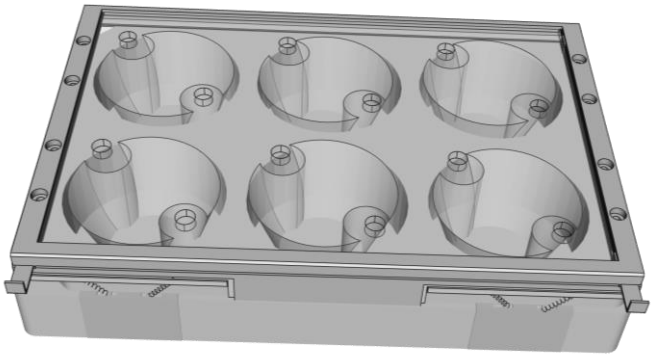
Be sure to have the marks aligned:



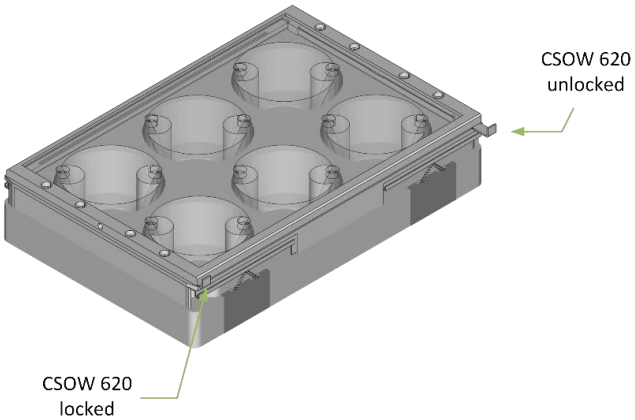
The tilted part of the plate holder should be facing the front when you assemble the lid and the holder.



Please be careful because the locking only workings if the plate is assembled in the good orientation. Make sure that the locking handles are open:

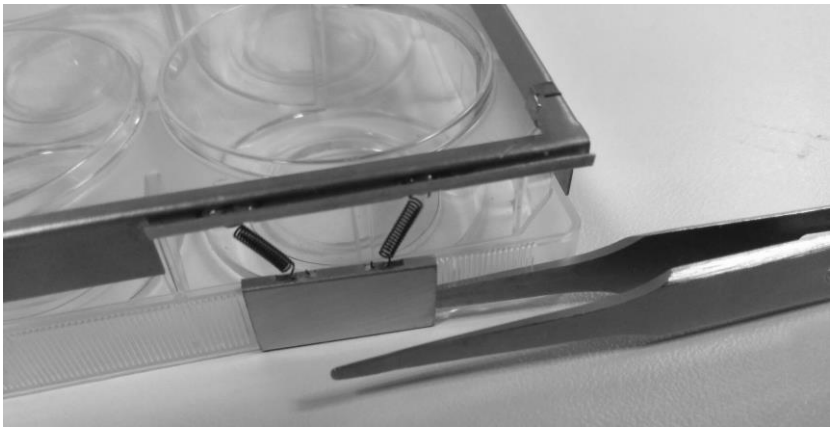


8. To confine cells, press the lid down on both left and right side and simultaneously lock the two handles. If you feel resistance make sure the plate is assembled in the good orientation.



9. To release the confinement, gently pull the locking handles out and let the lid come up. If it does not come up naturally, gently move it upwards.

10. If you have issues to remove the holding pads from the 6-well, you can use tweezers to help you remove it.



Cell Culture: The 6-well plate ca be coated with fibronectin or other ECM matrix of your choice. The cells can be plated some time before to enable them to adhere before being confined.