MultiWell Plate Development Kit

Nanomembrane-enabled barrier devices for cell culture

Product Nos. GASKET-MW

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INSTRUCTIONS FOR USE

Provided Kit Materials

- Pre-Cut Polydimethyl Silicone (PDMS) Gasket Set (suitable for 10 devices)

Required Equipment & Supplies

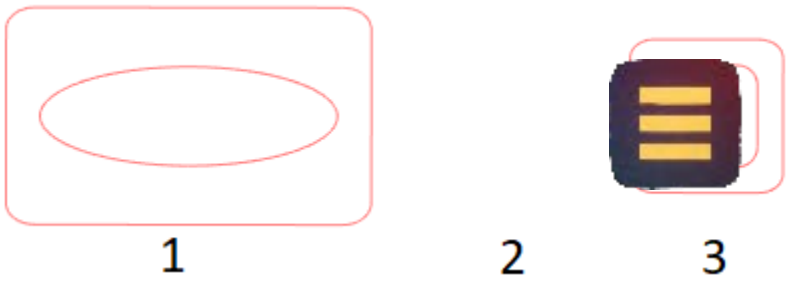
Equipment

- UV Ozone generator or Oxygen Plasma Oven
- Hot Plate or gravity oven

Supplies

- Tweezers suitable for membrane manipulation (SiMPore K6TWZR, or equivalent)
- User-supplied 24-well pre-sterilized Microplate
- Tray or substrate suitable for plasma or UV ozone activation
- Alcohol wipes or KimWipe saturated with an alcohol solution (70%)
- Absolute Alcohol (such as Isopropyl Alcohol (IPA) or Ethanol)
- Culture media (lab prepared or user supplied)
- Gloves and other suitable personal protective equipment (PPE)

Gasket Stack Layout



Layer #	Material
1 (Basal Well Layer)	1600 µm platinum cured PDMS
2 (Membrane Chip)	300 µm SiN/Si membrane chip
3 (Apical Well Layer)	1600 µm platinum cured PDMS

Figure 1. Gasket stack order of assembly

INSTRUCTIONS

Please read all **instructions** before proceeding. Refer to the gasket stack diagram above which details the order of assembly. If unfamiliar with assembling microfluidic

devices from PDMS, refer to the Tips & Troubleshooting section for more guidance.

Membrane Pre-Treatment (Optional but Recommended)

1. Plasma Cleaning of Membrane Chips for Cell Culture Applications
    - Working on a clean, well lit surface, remove the membrane chips to be used from the GelPack® shipping and storage container, and transfer to an appropriate substrate for plasma cleaning (e.g., ceramic or metal mesh tray)
    - Transfer the membrane containing substrate to the plasma cleaner and use instrument recommended settings for cleaning of glass or ceramic substrates. An example recipe may be found below.

        Power: 150 Watts
        Oxygen / Atmosphere flow: 30 SCCM
        Base Pressure: 200 mtorr
        Process Duration: 180 Seconds

    - Integrate within cell culture device immediately or store in inert containers (membrane side up, see Figure 2 below)

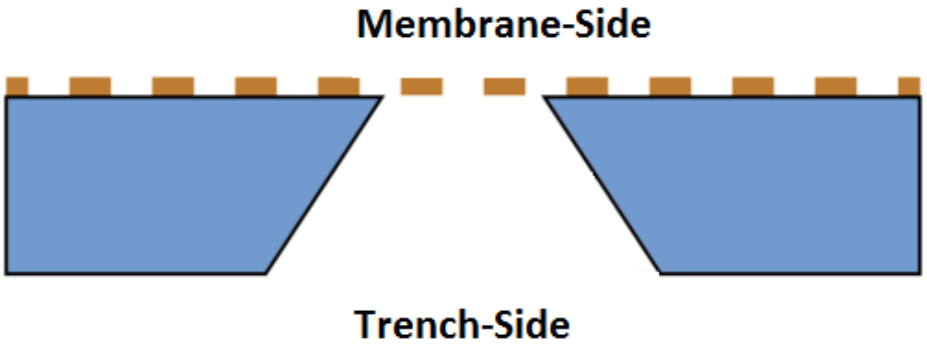


Figure 2. A representation of the two planar surfaces of SiMPore membrane chips. SiMPore membranes are deposed across the surface of a silicon wafer support material. Access to the membrane is achieve by etching trenches through the silicon wafer support, yielding the cross-sectional geometry shown above.

Device Assembly

1. Working on a clean, particle-free work surface, remove the Membrane Chip Gel pack, PDMS gaskets and multiwell plate from supplier packaging.
    - **NOTE:** It is highly recommended to pre-treat the membrane chips as above
    - **Recommended**: Sterilize the outside of each bag, then transfer to and perform this process in a biocabinet or laminar flow hood.
2. Using tweezers transfer the basal well layer (1), membrane chip (2), and apical well layer (3), and 24-well plate to the substrate. UV ozone or O2 Plasma source surface activate as per tool specifications.

NOTE: If assembling multiple wells, transfer the appropriate number of PDMS gasket and membrane components that may be assembled in ~ 180 seconds after surface activation

3. Using tweezers, carefully retrieve the now surface activated basal well layer (1) and gently place using a single motion in the bottom of a well, ensuring now air bubbles are trapped under the PDMS gasket

4. Using tweezers, carefully transfer and place a membrane chip centered over the basal well layer, pushing downwards slightly to expel trapped air.

   NOTE: Depending on application, the membrane chip may be placed with the membrane facing up or down. In either case the bonding process is the same. Refer to Tips and Troubleshooting for more details regarding membrane orientation in the device.

5. Continue the layer addition process by carefully transferring an apical well layer and applying it to the membrane surface using a single motion.

   NOTE: DO NOT ALLOW THE APICAL LAYER GASKET TO CONTACT THE MEMBRANE SURFACE

6. Complete assembly by firmly pressing down on the apical layer to promote adhesion of all device layers.

7. Repeat steps 2 through 6 for all membrane barrier devices to be built in the 24-well plate. A representative device image is shown below in Figure 3.

8. The device maybe cured at 50c for 2-24hrs to improve bonding strength between the layers

   NOTE: After curing the device surfaces may return to a hydrophobic state, making channel filling and the membrane pores difficult. A final plasma activation may be employed to enable complete filling of the basal well compartment

Using the Device

1. After curing and plasma activation the device may be sterilized with 70% IPA by completely filling the apical and basal chambers, then incubating for 10 minutes at room temperature

   NOTE: to ensure continued sterility handle the device only within a biocabinet or laminar flow hood from this point forward.

2. Following sterilization, aspirate the IPA solution to waste and wash both wells at least 3X with PBS or HBSS to displace the alcohol solution.

3. Finally, add cells to the appropriate layer in complete medium.

4. Infilling empty wells with deionized water, or the void spaces between wells, will create more locally humidified environment and prevent desiccation. However, due to the small well volumes daily checking of well volume and periodic refilling is recommended.

Table 1. Device Fill Volumes

Well	Max Volume (µL)	Recommended Volume (µL)
Apical	**22.5**	**20**
Basal	**50**	**45**

TIPS AND TROUBLESHOOTING

- **Inspection:** Prior to assembling device layers, carefully inspect the gaskets for debris, which may include PDMS cuttings or plugs (particularly in fluid ports or other small features). If debris is noted rinsed thoroughly with absolute alcohol or other solvent, then dry completely before use.
- **Cleanliness:** It is of critical importance to maintain the cleanliness of the PDMS surface to ensure proper bonding between gasket layers. If dust, debris, or other visible debris is present, attempt to remove via tweezers or compressed nitrogen gas. If accidental contact is made with an ungloved hand the PDMS gasket must be thoroughly cleaned with IPA or other solvent to remove any surface oil.
- **Surface Activation:** Surface activation of PDMS should be performed via UV Ozone or PDMS. As tool settings and operating parameters vary, development of an optimized recipe for complete surface activation is left to the user. Once surface activated the PDMS should be bonded as quickly as possible, within five minutes for optimal bonding results.
- **Proper Sealing:** Good bonding between the two PDMS layers is critical to ensure device performance. Debris or bubbles between the layers may cause device failure. Once bonded, no movement between the layers is advised (e.g. replacement during alignment), as the bond strength is greatly diminished with repeated contact. If misplacement occurs, repeat the surface activation before bonding again.
- **Curing:** Once fully assemble, the device must be heat-cured before use to improve the integrity of the bonded layers. Bake the assembled device on a hot plate or more ideally a gravity oven, for at least 120 minutes at 50ºC. Bake times will vary depending on the tool used. Do not exceed the bake temperature recommended by the Cultureware manufacturer.

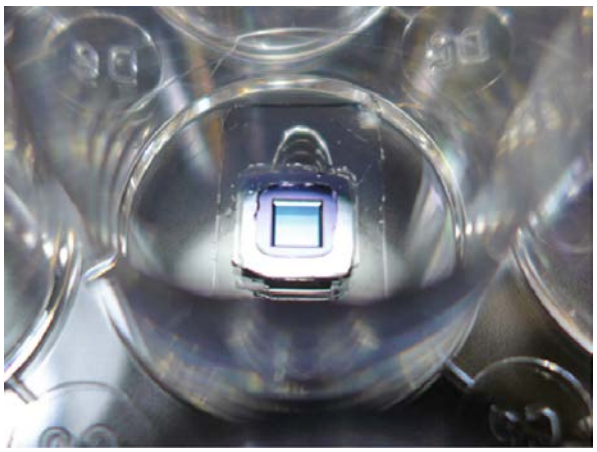


Figure 3 A fully assembled device shown in the bottom a well.

PHYSICAL PROPERTIES

Property	Value
Membrane Composition:	Silicon Nitride (SiN)
Membrane Surface Area	1.4 mm^2 (single slot), 6.3 mm^2 (three slot)
Minimum Working Length	2000 µm
Membrane Thickness:	100 nm (NPN) / 400 nm (SiN)
Pore Size Cut-Off:	varies
Porosity:	varies
Surface Charge:	Neutral to slightly negative
Wetted Components Material(s):	PDMS, Si, SiN, SS, PC/PS (MultiWell Plate)

SPECIFICATIONS

Sterilization

MultiWell devices may be sterilized via Ethylene Oxide, Hydrogen Peroxide Gas, 70% IPA immersion, or gamma, UV, and E-Beam irradiation after removing from the plastic bag. **Do Not** Sterilize via steam autoclave as device damage may occur.

Chemical Stability

Devices are incompatible with strong bases, as membrane degradation may occur. Avoid long exposure durations to solvents, as this may compromise device integrity. Do not apply solvents, acids, bases, organics, or other compounds that are incompatible with the stated device components.

Device Storage

Store in a clean and dry environment. Prolonged exposure to UV can degrade the PDMS bonding, leading to poor layer adhesion and shortened service life.

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TRADEMARKS AND PATENTS

Version 1226