

## Dynamic Cell Culture on a Pillar Plate using a Perfusion Plate

This standard operating procedure (SOP) provides step-by-step instructions for dynamic cell culture on the 36PillarPlate/144PillarPlate using the 36PerfusionPlate/144PerfusionPlate. Please read the protocol carefully before performing experiments.

### Materials:

- 36PillarPlate (Bioprinting Laboratories Inc., Cat. no. 36-01-00)
- 144PillarPlate (Bioprinting Laboratories Inc., Cat. no. 144-01-00)
- 36PerfusionPlate (Bioprinting Laboratories Inc., Cat. no. 36-02-00)
- 144PerfusionPlate (Bioprinting Laboratories Inc., Cat. no. 144-02-00)
- 36PetriLid (Bioprinting Laboratories Inc., Cat. no. 36-03-00)
- 144PetriLid (Bioprinting Laboratories Inc., Cat. no. 144-03-00)
- Deep petri dish, 100 mm x 20 mm (Corning, Cat. no. 70165-102)
- OrganoFlow L Digital Rocker (MIMETAS) or RoboRocker™ 210 series (Next Advance)
- Vacusafe vacuum aspiration system (Integra Biosciences, Part no. 158320)

### Methods:

1. Set the digital rocker to a 10° tilt angle (5° per second) with a 1-minute rocking interval to generate bidirectional flow in the perfusion plate (**Fig. 1**).

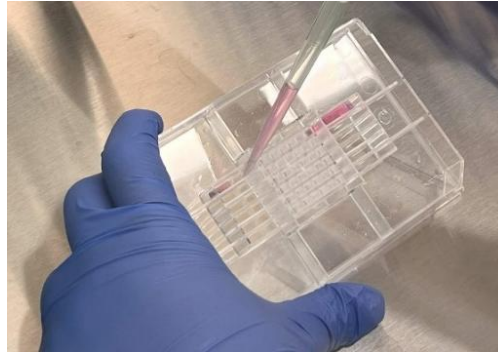
**Note:** *The tilt angle influences the flow rate in the perfusion plate, whereas the interval is determined by the time necessary to drain the cell growth medium in the upper reservoirs in the perfusion plate. With a higher tilt angle, a shorter interval is required. If low-shear conditions are required to minimize spheroid detachment from the pillar plate, set the digital rocker to a 5° tilt angle with a 5-minute rocking interval.*



**Figure 1.** OrganoFlow L digital rocker for loading multiple perfusion plates.

2. Dispense 400  $\mu$ L of a cell growth medium in each reservoir of a 36PerfusionPlate (800  $\mu$ L cell growth medium per fluidic channel) and place it on a flat surface for 1 - 2 minutes to fill all perfusion wells with the cell growth medium (**Fig. 2**). For a 144PerfusionPlate, dispense 800  $\mu$ L of a cell growth medium in each reservoir (1600  $\mu$ L cell growth medium per fluidic channel). **Note:** Adding an excessive volume of cell culture medium to the perfusion plate can cause overflow after the pillar plate is sandwiched and the assembly is placed on the digital rocker. Avoid wetting the bottom of the pillars with cell culture medium, as it can lead to cross-talk or

*contamination during dynamic cell culture. If any perfusion wells are not filled with cell growth medium, manually dispense 60  $\mu$ L of the medium in the empty perfusion wells.*

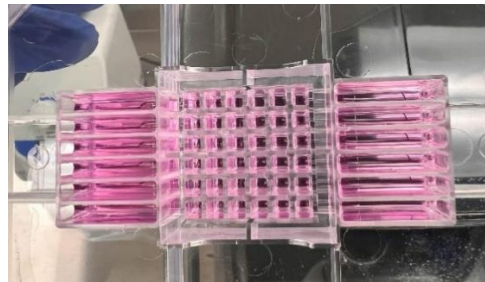


**Figure 2.** Manual dispensing of a cell growth medium in reservoirs of the 36PerfusionPlate.

3. Cover the 36/144PerfusionPlate with a lid and place it on the digital rocker in a humidified 5% CO<sub>2</sub> incubator at 37°C for 30 minutes.

**Note:** *Ensure uniform flow of the cell growth medium in the perfusion plate by verifying the medium levels in the upper and lower reservoirs as well as the perfusion wells. Pre-warming the cell growth medium helps minimize bubble formation on the 36/144PillarPlate after loading cells or spheroids in hydrogel.*

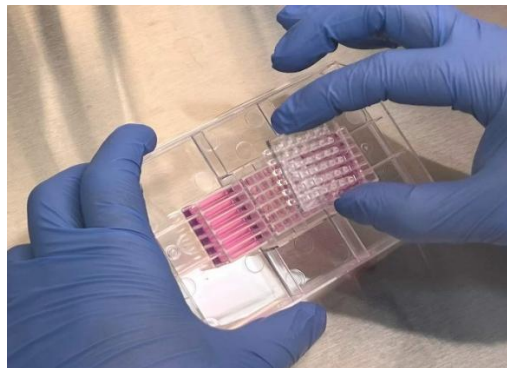
4. Take the perfusion plate out of the incubator and check the level of the cell growth medium in upper and lower reservoirs as well as perfusion wells (**Fig. 3**).



**Figure 3.** Uniform level of the cell growth medium in the 36PerfusionPlate.

5. Sandwich the pillar plate with cells/spheroids onto the perfusion plate and cover the sandwiched plates with a lid (**Fig. 4**).

**Note:** *For cell/spheroid loading on the 36/144PillarPlate, refer to the relevant SOPs titled “Spheroid Culture on Pillar Plate” and “Cell Suspension Culture on Pillar Plate”.*



**Figure 4.** Sandwiching the 36PillarPlate with cells/spheroids onto the perfusion wells of the 36PerfusionPlate for dynamic cell culture.

- Inspect the sandwiched plates under a brightfield microscope to ensure uniform cell loading on the entire pillar plate.
- Place the sandwiched plates on the digital rocker in a humidified 5% CO<sub>2</sub> incubator at 37°C and perform dynamic cell culture with medium changes every 2 days (**Fig. 5**).

**Note:** Ensure uniform flow of the cell growth medium in the perfusion plate from the sideview.

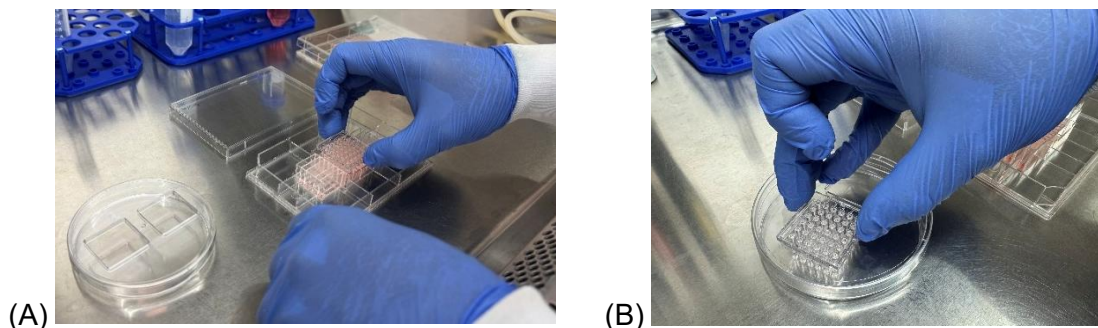


**Figure 5.** Dynamic cell culture on the 144PillarPlate with the 144PerfusionPlate on the digital rocker at a 10° tilt angle with a 30-second rocking interval.

#### Medium replacement procedure

- To replace the old cell culture medium, carefully separate the pillar plate containing cells from the perfusion plate, and immediately insert the pillar plate into the 36PetriLid (or 144PetriLid) positioned on a 100 x 20 mm petri dish containing 5 mL of sterile distilled water (**Fig. 6**).

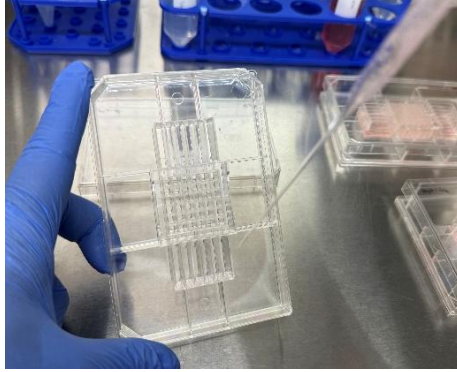
**Note:** Do not place the wet pillar plate directly on a flat surface. This causes cell culture medium to drip from the pillar tip to the bottom of the pillar. If the bottom of the pillar becomes wet, it can lead to “overflow” when the pillar plate is reinserted into the perfusion plate. Therefore, always keep the pillar plate inserted in the PetriLid during medium replacement to prevent pillar bottom wetting and minimize evaporation of residual moisture.



**Figure 6. (A)** Separation of the 36PillarPlate from the 36PerfusionPlate. **(B)** Insertion of the 36PillarPlate into the 36PetriLid placed on a 100 x 20 mm petri dish to maintain the pillars in a downward orientation and prevent wetting of the pillar bottoms during medium replacement.

- While holding the perfusion plate at approximately a 45° angle, allow the old cell culture medium to drain toward the lower reservoirs. Aspirate and remove the medium completely from the far end of the lower reservoirs using a Vacusafe vacuum aspiration system (Integra Biosciences) (**Fig. 7**).

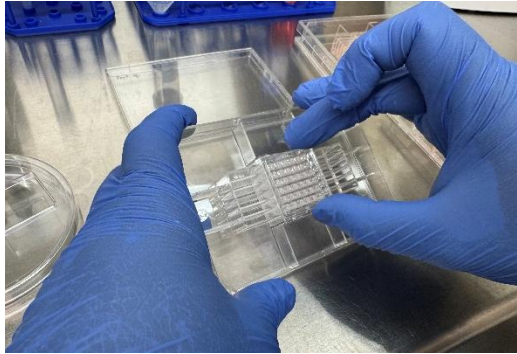
**Note:** Ensure complete removal of the old medium from all perfusion wells and reservoirs to prevent dilution of freshly added medium and accumulation of waste metabolites.



**Figure 7.** Vacuum aspiration of the old medium from the far end of the lower reservoirs.

- Carefully remove the pillar plate from the 36PetriLid (or 144PetriLid) and reinsert it onto the corresponding perfusion plate (**Fig. 8**).

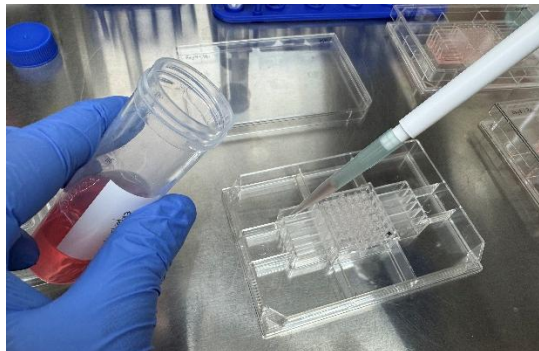
**Note:** Before insertion, ensure that all pillars are properly aligned with the perfusion wells. Misalignment may cause mechanical disturbance of the cultured cells on the pillars.



**Figure 8.** Reinserting the pillar plate onto the perfusion plate after old medium removal.

- Slowly dispense 400  $\mu\text{L}$  of fresh, warm cell growth medium into each reservoir of the 36PerfusionPlate (for a total of 800  $\mu\text{L}$  per fluidic channel) (**Fig. 9**). For the 144PerfusionPlate, dispense 800  $\mu\text{L}$  into each reservoir (1,600  $\mu\text{L}$  per fluidic channel).

**Note:** Dispense the fresh, pre-warmed cell growth medium from the far end of the reservoirs (away from the microchannels) to prevent overflow in the perfusion wells caused by capillary action.



**Figure 9.** Dispensing fresh, pre-warmed cell growth medium from the far end of each reservoir in the 36PerfusionPlate to prevent capillary-driven overflow into the perfusion wells.

- Leave the sandwiched plates on a flat surface for approximately 1 minute to allow the medium to equilibrate and distribute evenly across all perfusion wells and channels.
- Place the sandwiched plates containing fresh cell growth medium onto the digital rocker, and resume dynamic cell culture inside the  $\text{CO}_2$  incubator.