1. Objective

This protocol describes the procedure for medium changes of cultures grown in the OrganoPlate® 2-lane and 3-lane.

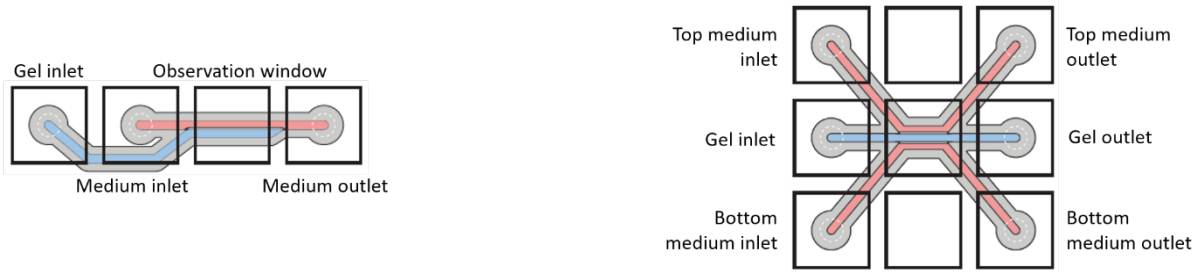


***Figure 1**: Schematic representation of a tissue chip from OrganoPlate® 2-lane (left) and 3-lane (right)*

2. Background

Most cultures in the OrganoPlate® require medium refreshment every 2-3 days. Old medium can be aspirated using a multichannel aspirator system. After the wells of medium inlets and outlets are emptied, fresh medium can be added using a multichannel repeating pipette. The procedures described in this protocol allow for medium changes in OrganoPlate® 2-lane and 3-lane and can also be used for other assays, such as fixation.

3. Materials

- OrganoPlate® 2-lane or 3-lane with cells
- Cell specific medium
- Multichannel aspirator system
- Aspirator tips
- Multichannel repeating pipette (e.g. Sartorius, 16015143)
- Pipette tips (e.g. Sartorius, 791201, 50-1200 µL tips)
- Multi-channel medium reservoir (e.g. VWR, 89094-680)

4. Procedure

Aspiration

The procedure below applies to both OrganoPlate® 2-lane and 3-lane

1. Remove the lid from the OrganoPlate®
2. Aspirate medium from medium inlets and outlets using a multichannel aspirator

a. Place the tips in the corner of the well and allow them to touch the bottom of the plate
b. Placing the tips immediately on top of the holes of the microfluidics should be avoided as this may disturb the culture or cause the formation and trapping of air bubbles

Medium addition

OrganoPlate® 2-lane

1. Take up medium from a medium reservoir using a multichannel repeating pipette
2. Dispense medium in all medium inlets and medium outlets (i.e. all wells in columns 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24)
   a. Dispense the medium against the wall of a well to prevent air bubble formation
   b. Dispense first in inlets from column 2, rows A, C, E, G, I, K, M, O (pipette tips of an 8-tip multichannel pipette will align with these wells naturally)
   c. Move the pipette down and dispense in remaining inlets from column 2 (rows B, D, F, H, J, L N, P)
   d. Repeat for the medium outlets of this set of chips (column 4)
   e. Repeat procedure for remaining medium inlets (columns 6, 10, 14, 18, 22) and medium outlets (columns (8, 12, 16, 20, 24)
   f. See figure 2

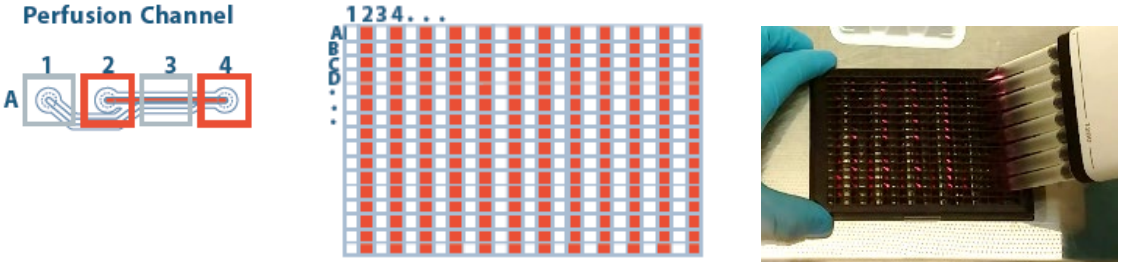


***Figure 2**: Medium addition in OrganoPlate® 2-lane. Medium inlets and outlets are indicated in red.*

OrganoPlate® 3-lane

1. Take up medium using a multichannel repeating pipette from a medium reservoir
2. Dispense medium in all medium inlets and medium outlets according to the pipetting scheme shown in figure 3 on the next page.

5. Proceed with culture

1. Place the OrganoPlate® back (on the OrganoFlow®) in a humidified incubator
   a. The use of OrganoFlow® depends on the type of culture
2. Proceed with culture

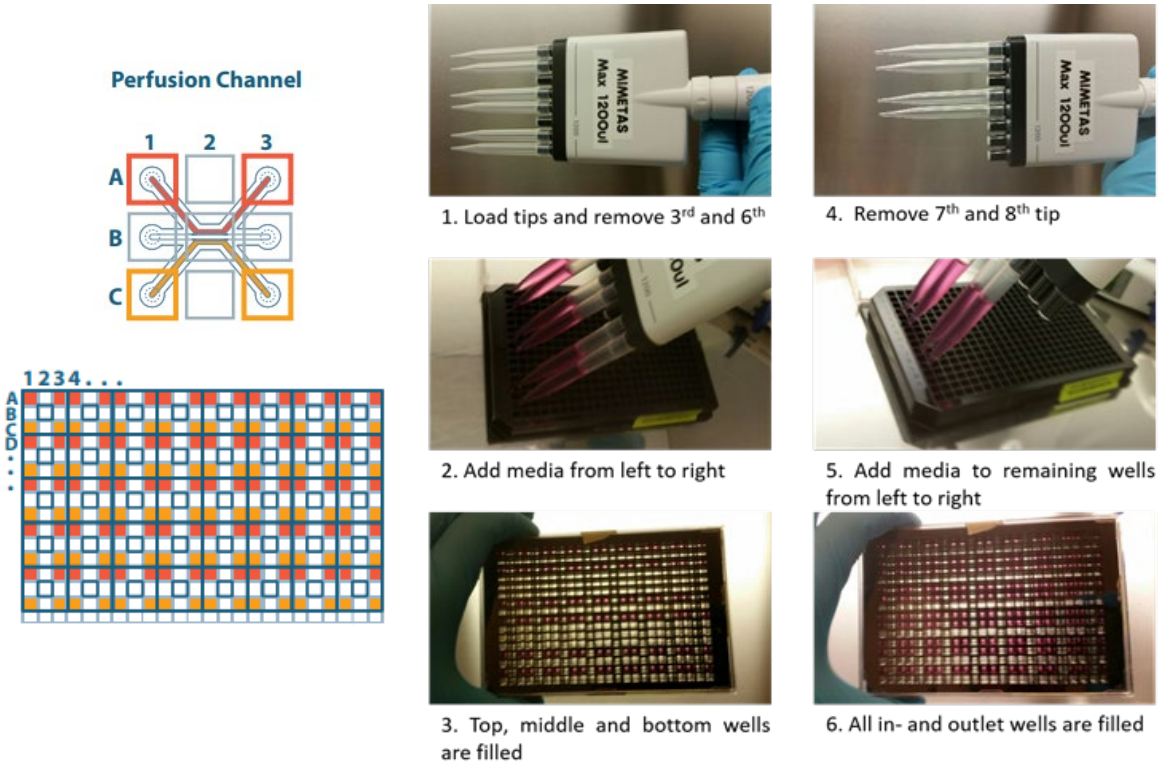
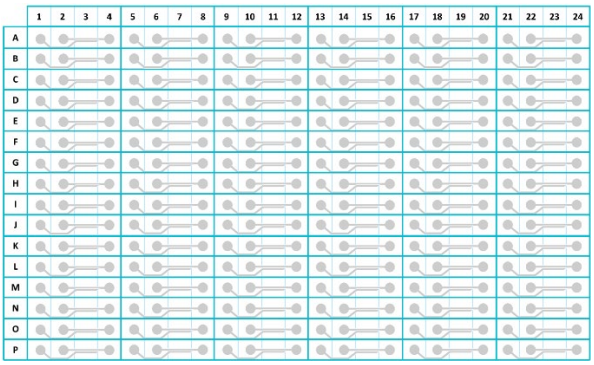


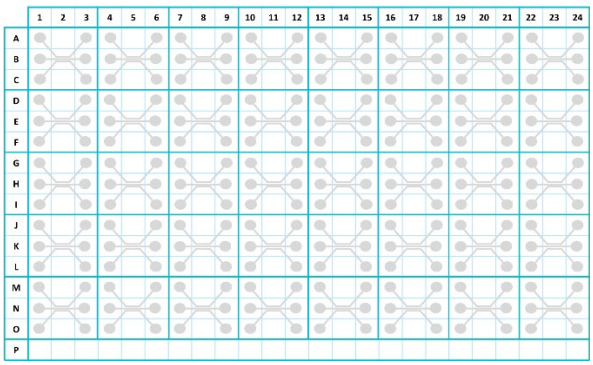
***Figure 3**: Medium addition to OrganoPlate® 3-lane. Medium inlets and outlets are indicated in red and yellow.*

6. Plate layouts

OrganoPlate® 2-lane



OrganoPlate® 3-lane



MIMETAS product list

Cat. No.	Product Name
MI-AR-CC-01	OrganoReady® Caco-2
9605-400-B	OrganoPlate® 2-lane
4004-400-B	OrganoPlate® 3-lane 40
6405-400-B	OrganoPlate® 3-lane 64
6401-400-B	OrganoPlate® Graft
MI-OFPR-S	OrganoFlow® S
MI-OFPR-L	OrganoFlow® L
MI-OT-1	OrganoTEER®

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