Barrier Integrity Assay in the OrganoPlate®

1. Objective

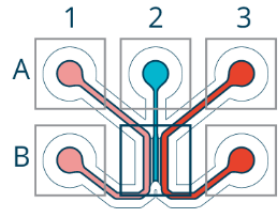
This assay is performed to assess the barrier integrity and Papp of a barrier tissue and can be used for the following applications:

- For quality control purposes before and/or after a transcytosis assay.
- To quantify compound-induced disruption of the barrier (endpoint or in real-time)

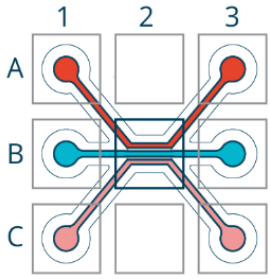
For further information please contact us at support@mimetas.com or consult Soragni et al 2023.

2. Background

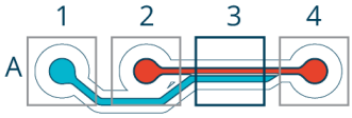
In the OrganoPlate®, cells can be seeded against an ECM gel to establish tubular structures, such as endothelial or epithelial barrier tissues. These tissues form adherens and tight junctions and can form a leak tight monolayer. The tightness of the monolayer can be assessed with the barrier integrity assay. For this assay, the regular culture medium is replaced by medium containing a fluorescent dye and leakage of the dye from the perfusion channel, which represents the lumen of the tubule, into the adjacent ECM gel is monitored. The barrier integrity assay is compatible with both the OrganoPlate® 2-lane and 3-lane.



OrganoPlate® 3-lane 64



OrganoPlate® 3-lane 40



OrganoPlate® 2-lane

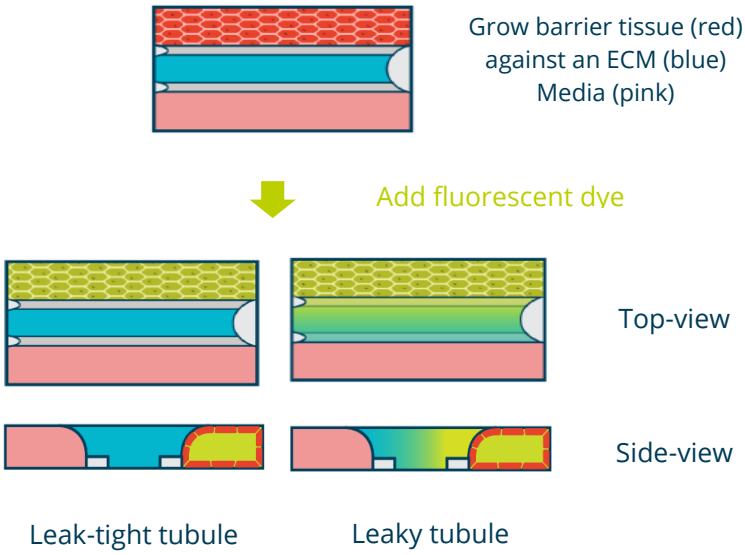


Figure 1: *Schematic overview of the barrier integrity assay in the OrganoPlate® 3-lane*

3. Materials

(keep all solutions sterile)

- OrganoPlate® 3-lane or 2-lane with barrier tissue (i.e. epithelial tubules)
- Cell-type specific culture medium
- Fluorescent dyes
    a) Examples of commonly used fluorescent dyes include:
        i. TRITC-dextran 155 kDa (Sigma, T1287, in HBSS)
        ii. FITC-dextran 150 kDa (Sigma, 46946, in HBSS)
        iii. FITC-dextran 20 kDa (Sigma, FD20S, in HBSS)
        iv. FITC-dextran 10 kDa (Sigma, FD10s, in HBSS)
        v. TRITC-dextran 4.4 kDa (Sigma, T1037, in HBSS)
        vi. Sodium Fluorescein (Sigma, F6377, in HBSS)

4. Assay

4.1 *Set the microscope to allow image acquisition to start as soon as dyes are added to the chips*

4.2 Prepare a fluorescent working solution by mixing one, or more differently labelled tracing dyes together. The final working concentration of the fluorophore dyes is generally 0.5 mg/mL each (10µg/ml for Sodium Fluorescein), into normal culture media, for example:

- Caco-2 media for Caco-2 tubules
- OrganoMedium HBMEC-BM for HBMEC tubules
- OrganoMedium HUVEC-BM for HUVEC tubules

Every chip of any OrganoPlate® format requires 70 µL fluorophore media.
For example, a typical working solution of 0.5 mg/mL TRITC-dextran 155 kDa and 0.5 mg/mL FITC-dextran 20 kDa for 1* OrganoPlate® of 64 chips would be prepared as following:

i. Add 4.8 mL medium to a 15 mL tube
ii. Add 100 µL of TRITC-dextran 155 kDa (25 mg/ml stock solution)
iii. Add 100 µL of FITC-dextran 20 kDa (25 mg/ml stock solution)

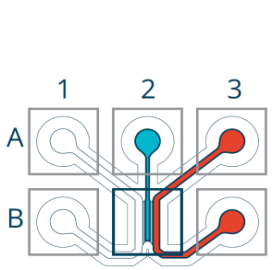
4.3 A cell-specific solution is to be added into the opposite basolateral channel. This is either cell culture media or HBSS (supplied with OrganoReady®). For example:

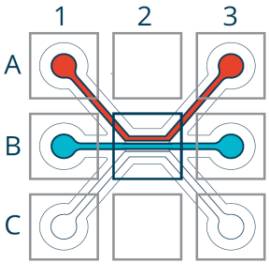
- Caco-2 media for Caco-2 tubules
- HBSS$^{Ca2+/Mg2+}$ for HUVEC and HBMEC tubules

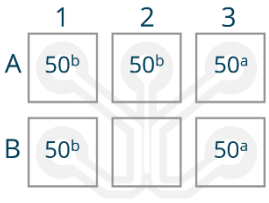
As detailed in step 4.6 below, each OrganoPlate® type requires a different volume of solution per chip. 20 µL, 80 µL and 60 µL for the 2-lane, 3-lane 40 and 64 respectively.

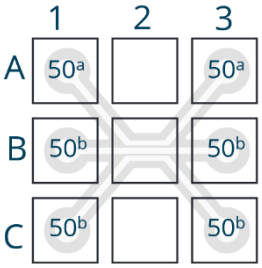
4.4 Wetting: (optional step).

Omit and proceed immediately to step 4.5 if you are working with an OrganoPlate® 3-lane, in either 40 or 64 chips layout, in which all channels are perfused with media. If there is no liquid present in the opposite channels to the tubule, and there is no medium in the respective inlets and outlets, it is essential to first perform a "wetting" step before starting the BI assay, to ensure proper flow profiles and successful readouts as follows:



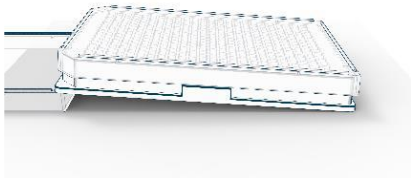






NOTE: The medium should be pipetted into all dry channels. This protocol details for cell tubules grown on the top or right perfusion channels (in red) and should be adjusted accordingly with different cell culture layouts. Empty lanes are indicated in white, ECM in blue.

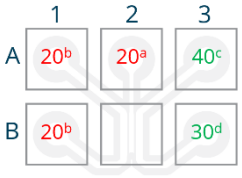
a. Leave 50 µL of medium in the tubule inlets and outlets
b. Add 50 µL medium to all remaining inlets and outlets
c. Place plate under an angle and perfuse for 5 minutes (e.g. by placing one side on a lid, see image on the right)



4.5 Aspirate all medium from inlets (first) and outlets (second)

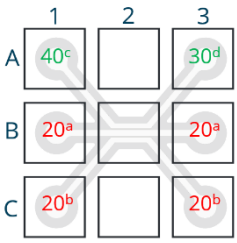
4.6 Add the following solutions in the following specific order:

OrganoPlate® 3-lane 64 for tubules grown on the right channel



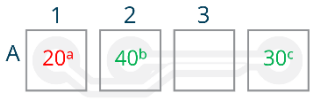
i. Pipette 20 µL of solution in the gel inlet
ii. Pipette 20 µL of solution in the left inlet and outlet
iii. Pipette 40 µL of the fluorophore media in the right medium inlet
iv. Pipette 30 µL of the fluorophore media in the right medium outlet
v. Proceed to image acquisition.

OrganoPlate® 3-lane 40, for tubules grown in the top channel:



i. Pipette 20 µL of solution in the gel inlet and outlet
ii. Pipette 20 µL of solution in the bottom inlet and outlet
iii. Pipette 40 µL of the fluorophore media in the top medium inlet
iv. Pipette 30 µL of the fluorophore media in the top medium outlet
v. Proceed to image acquisition.

OrganoPlate® 2-lane



i. Pipette 20 µL of solution in the gel inlet
ii. Pipette 40 µL of the fluorophore media in the medium inlet
iii. Pipette 30 µL of the fluorophore media in the medium outlet
iv. Proceed to image acquisition.

5. Image acquisition

This assay can be performed using a (high-content) fluorescent microscope. The exact protocol for your specific imaging system may require optimization. Keep the following points in mind when imaging the barrier integrity assay:

5.1 Make sure the dye is in focus when imaging. Image-based focusing algorithms can struggle with large fronts of dye. An alternative here is to turn off image-based auto-focusing and pre-define a single focal point (also known as Z-location). Laser-based focusing systems usually have no issues with this assay as they are unaffected by the presence of dye inside the channel.

5.2 Make sure not to over-expose the fluorescent dyes when setting the exposure times. Saturated signals will hamper correct quantification of the assay in a later stage.

5.3 You can choose to do an end-point measurement only, or to monitor barrier integrity over time. To do the latter, image each chip at several set time points. Depending on the speed of the microscope, you can for example image each chip of an OrganoPlate® 3-lane every 2 minutes or each chip of an OrganoPlate® 2-lane every 4 minutes for the total duration of the assay (i.e. 30 minutes).

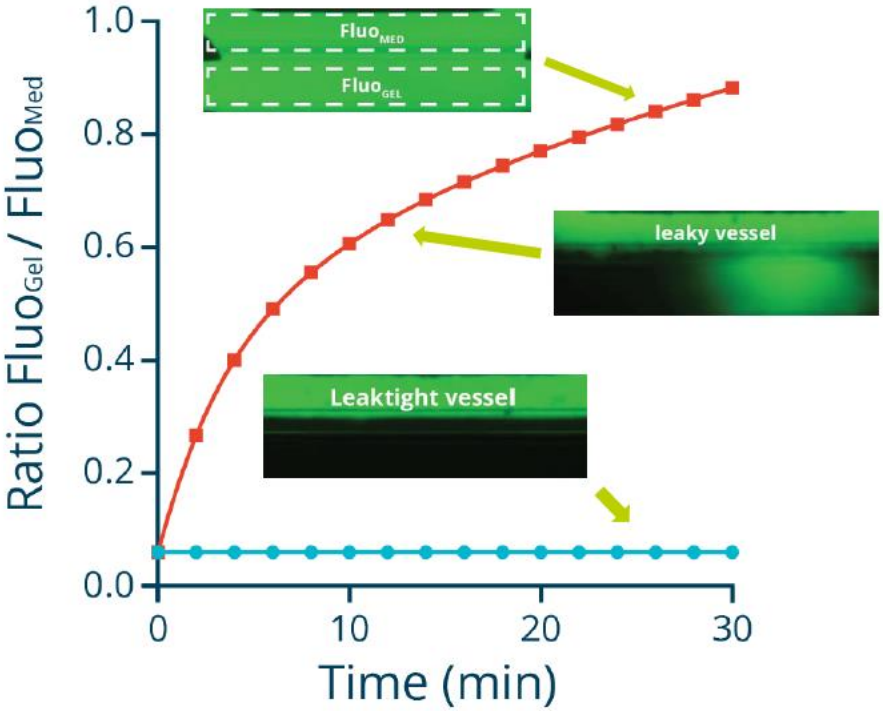


Figure 2: *Example of quantification of the barrier integrity assay in the OrganoPlate®. In case of a leak-tight barrier, the ratio of fluorescent signal measured in the medium channel and the gel channel remains constant and is relatively low (blue line). In case of a leaky barrier or a cell-free control, the ratio increases over time and eventually approaches one (red line).*

6. Optional: continue culture

To continue culture of the tubules after performing the barrier integrity assay, aspirate all solutions from the wells and add 50 µL of fresh culture medium to all perfusion inlets and outlets. Then place the OrganoPlate® back on the OrganoFlow® in the incubator to continue culture.

7. Optional: inducing barrier disruption

Barrier disruption can be induced in barrier models grown in the OrganoPlate, e.g. by exposure to experimental toxicants. The table below provides examples of barrier disrupting conditions for various tissues grown in the OrganoPlate®.

Tissue	Exposure	Duration	Expected effect	Reference
Caco-2	Staurosporine, 0.5 µM	24 h	Mild barrier disruption	Publication
Caco-2	Staurosporine, 90 µM	24 h	Severe barrier disruption	Publication
RPTEC	Cisplatin, 30 µM	48 h	Mild barrier disruption	Publication
RPTEC	Cisplatin, 250 µM	48 h	Severe barrier disruption	Publication
HBMEC	Staurosporine, 0.033 µM	24 h	Mild barrier disruption	Publication
HBMEC	Staurosporine, 0.1 µM	24 h	Severe barrier disruption	Publication

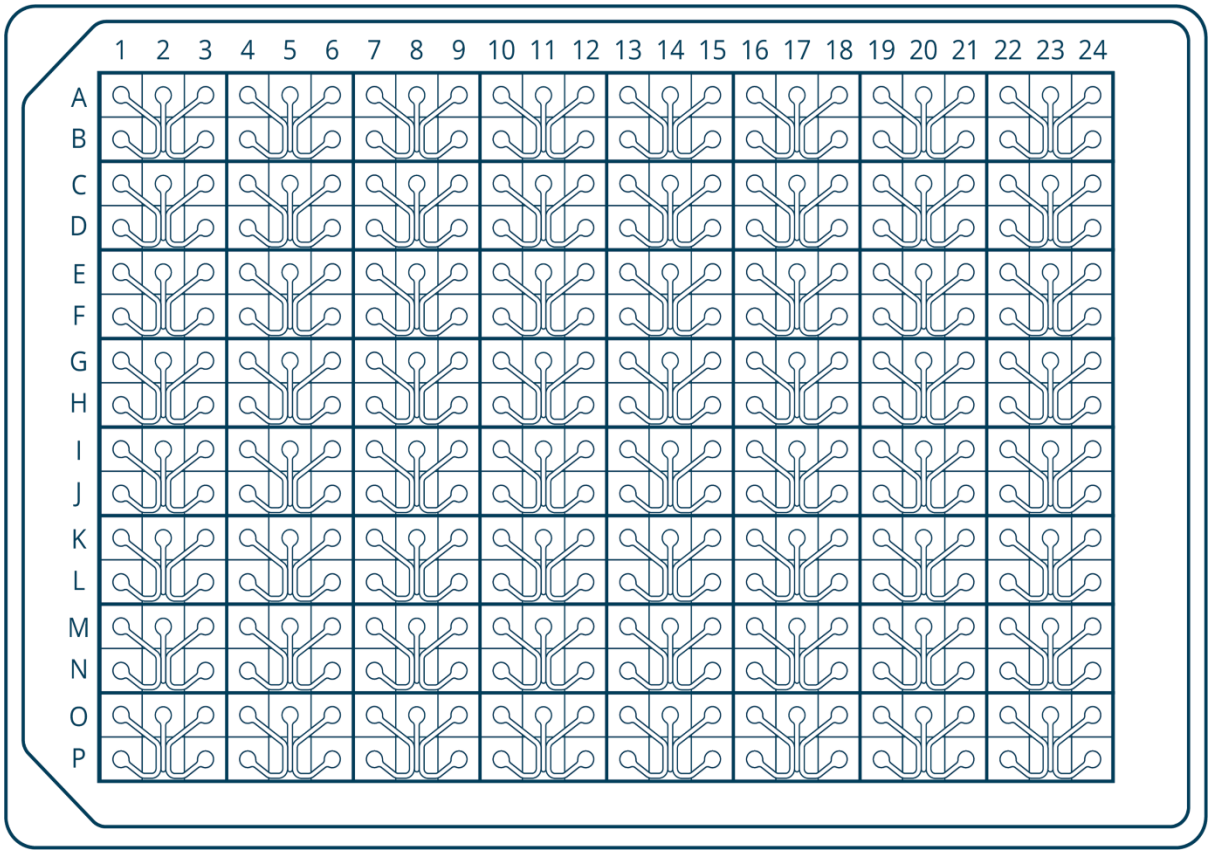
8. Data analysis

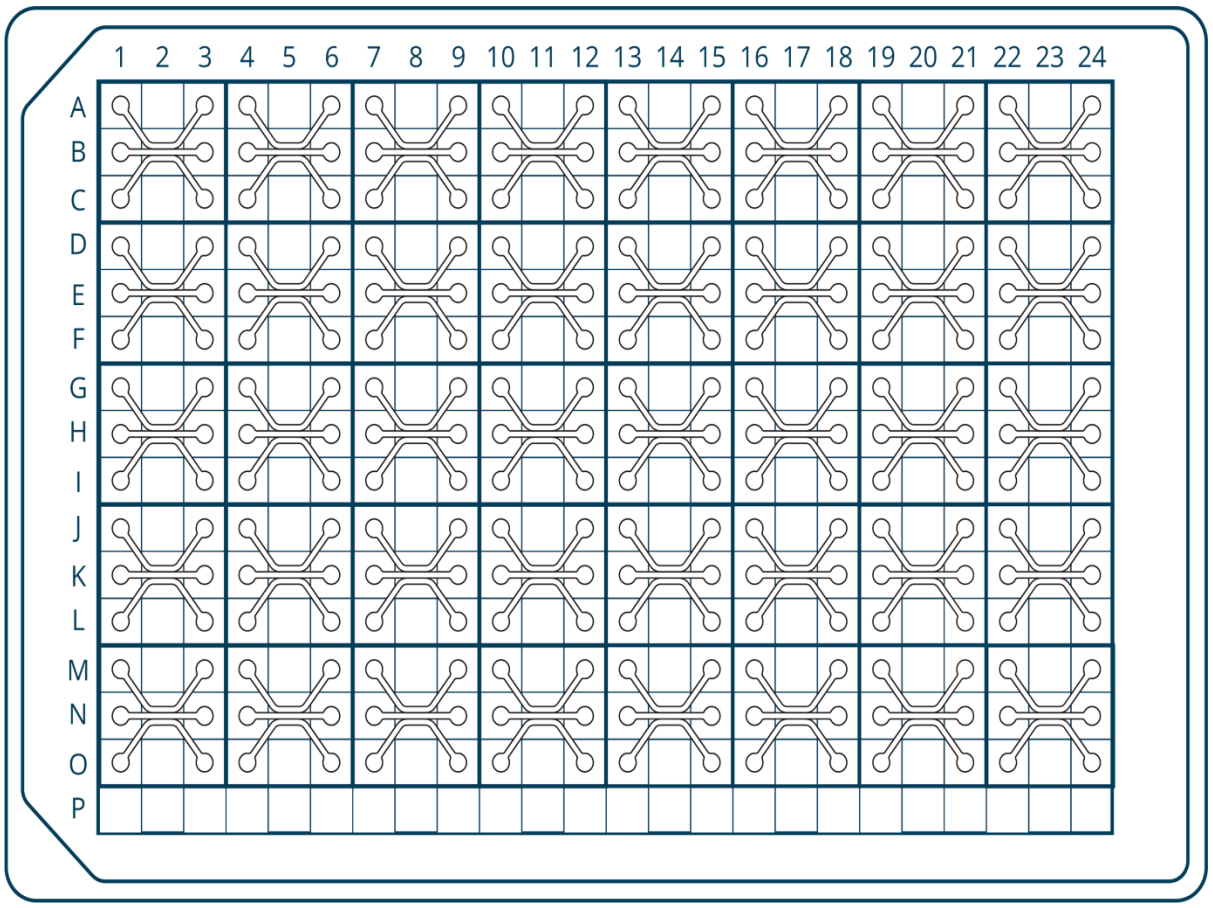
Integrity of a barrier tissue in the OrganoPlate® can be quantified using the following approach.

Determine the ratio of fluorescent signal in the perfusion channel (which represent the lumen) and the adjacent gel channel of a chip, at endpoint or over time.

a. In case the barrier tissue is leak tight, this ratio will remain constant and is relatively low (because the fluorescent signal in the perfusion channel is very high, while the fluorescent signal in the gel channel is very low).
b. In case the barrier tissue is leak tight, this ratio will remain constant and is relatively low (because the fluorescent signal in the perfusion channel is very high, while the fluorescent signal in the gel channel is very low).
c. Figure 2 exemplifies the quantification of the barrier integrity assay

Plates layout

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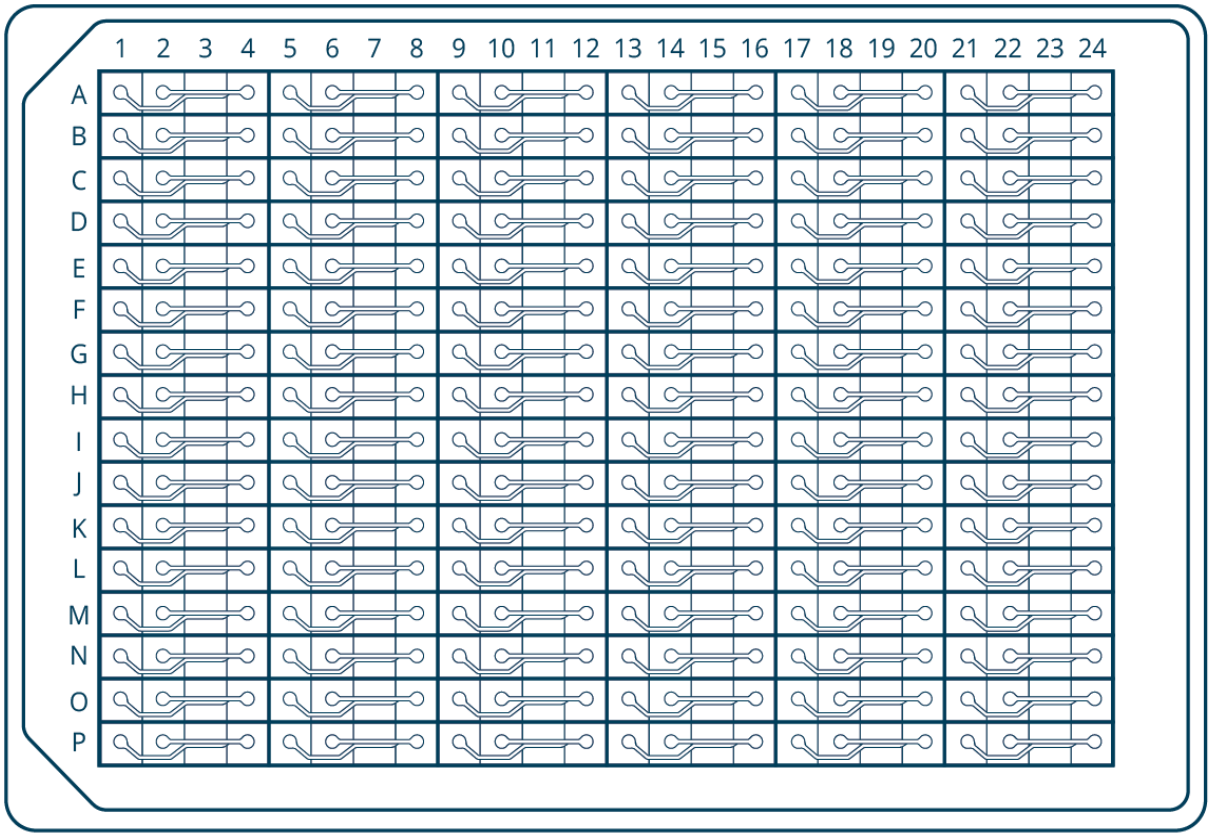
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MIMETAS Product List

Cat. No.	Product Name
MI-OR-CO-CU -01	OrganoReady® Collagen 3-lane 40
MI-OR-CO-CU-02	OrganoReady® Collagen 3-lane 64
MI-OR-CC-01	OrganoReady® Colon Caco-2 3-lane 40
MI-OR-CC-02	OrganoReady® Colon Caco-2 3-lane 64
MI-OR-BV-01	OrganoReady® Blood Vessel HUVEC 3-lane 40
MI-OR-BV-02	OrganoReady® Blood Vessel HUVEC 3-lane 64
MI-OR-AN-01	OrganoReady® Angiogenesis HUVEC 3-lane 64
MI-OR-HB-01	OrganoReady® BBB HBMEC 3-lane 40
MI-OR-HB-02	OrganoReady® BBB HBMEC 3-lane 64
MI-OM-BVBM-01	OrganoMedium® HUVEC-BM
MI-OM-HBBM-01	OrganoMedium® HBMEC-BM
MI-OFPR-S	OrganoFlow® S
MI-OFPR-L	OrganoFlow® L
MI-OT-VP2	OrganoTEER® Standard package

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