Barrier Integrity Assay in the OrganoPlate®

1. Objective

This assay is performed to assess the barrier tightness of a barrier tissue and can be used:

- As validation criteria for tissue modelling
- To select for leak tight tissues for a transport or toxicant exposure study
- To detect compound-induced disruption of the barrier (endpoint or in real-time)

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.

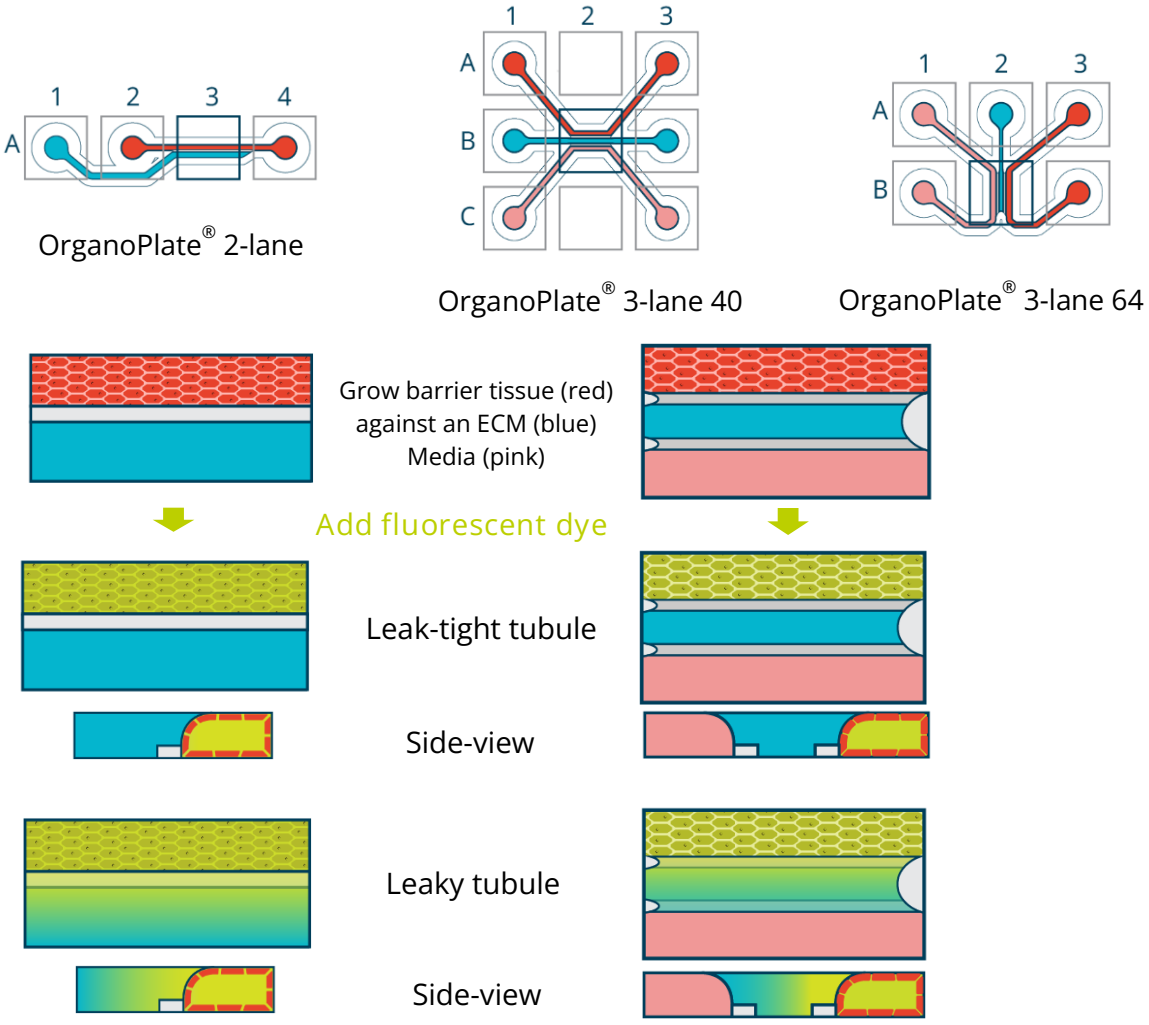


***Figure 1**: Schematic overview of the barrier integrity assay in the OrganoPlate® 2-lane and 3-lane*

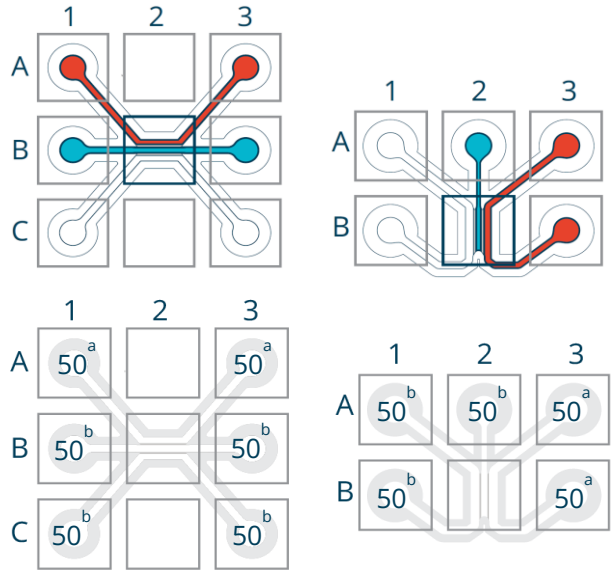
3. Materials

(keep all solutions sterile)

- OrganoPlate® 2-lane or 3-lane with barrier tissue (i.e. epithelial tubules)
- Cell-type specific culture medium
- Fluorescent dyes
    a) Commonly used fluorescent dyes include:
        i. TRITC-dextran 155 kDa (Sigma, T1287, stock solution 25 mg/mL in HBSS)
        ii. FITC-dextran 150 kDa (Sigma, 46946, stock solution 25 mg/mL in HBSS)
        iii. FITC-dextran 20 kDa (Sigma, FD20S, stock solution 25 mg/mL in HBSS)
        iv. FITC-dextran 10 kDa (Sigma, FD10s, stock solution 25 mg/mL in HBSS)
        v. TRITC-dextran 4.4 kDa (Sigma, T1037, stock solution 25 mg/mL in HBSS)

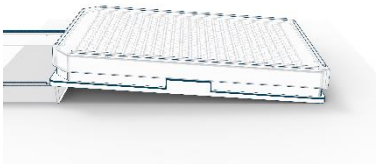
4. Assay

1. Set the microscope to allow image acquisition to start as soon as dyes are added to the chips
2. Prepare a fluorescent working solution containing one or more fluorescent dyes
    a) You can assess barrier integrity for several dyes of different sizes in one chip by mixing two differently labelled tracing dyes together in the fluorescent working solution
    b) The final concentration of the fluorescent dyes is 0.5 mg/mL each, requiring a 50x dilution from stock
    c) An OrganoPlate® 2-lane requires 70 µL working solution and 20 µL medium per chip
    d) An OrganoPlate® 3-lane requires 70 µL working solution and 80 µL medium per chip
    e) For example:
        i. Add 9.6 mL medium to a 15 mL tube
        ii. Add 200 µL of TRITC-dextran 155 kDa stock solution
        iii. Add 200 µL of FITC-dextran 20 kDa stock solution
        iv. Final fluorescent working solution contains 0.5 mg/mL TRITC-dextran 155 kDa and 0.5 mg/mL FITC-dextran 20 kDa
    3. **Omit step 3** and proceed immediately to step 4 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:



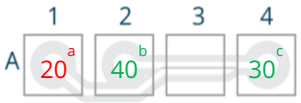
NOTE: The medium should be pipetted into all dry channels. This protocol details for cell tubules grown on the top or right perfusion channels (in red) and should be adjusted accordingly with different cell culture layouts. Empty lanes are indicated in white, ECM in blue.

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 an object, see image on the right)
d) Aspirate medium from all inlets and outlets
e) Proceed to step 5



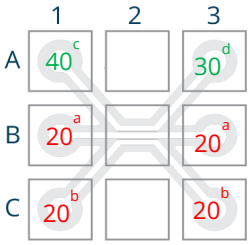
4. Start assay by pipetting the following solutions in this specific order:

OrganoPlate® 2-lane



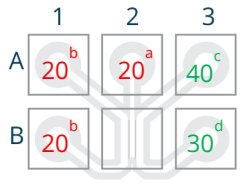
a. Pipette 20 µL of culture medium in the gel inlet
b. Pipette 40 µL of the working solution in the medium inlet
c. Pipette 30 µL of the working solution in the medium outlet
d. Proceed to image acquisition

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



a. Pipette 20 µL of culture medium in the gel inlet and outlet
b. Pipette 20 µL of culture medium in the bottom inlet and outlet
c. Pipette 40 µL of the working solution in the top medium inlet
d. Pipette 30 µL of the working solution in the top medium outlet
e. Proceed to image acquisition

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



a. Pipette 20 µL of culture medium in the gel inlet
b. Pipette 20 µL of culture medium in the left inlet and outlet
c. Pipette 40 µL of the working solution in the right medium inlet
d. Pipette 30 µL of the working solution in the right medium outlet
e. Proceed to image acquisition

5. Image acquisition

This assay can be imaged 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:

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.

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.

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).

6. Data analysis

1. Integrity of a barrier tissue in the OrganoPlate® can be quantified using the following approach.
2. 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 leaky, this ratio will increase over time, eventually approaching 1 (because the fluorescent signal in the gel channel is increasing as dye leaks in from the perfusion channel over time).
    c. Figure 2 exemplifies the quantification of the barrier integrity assay

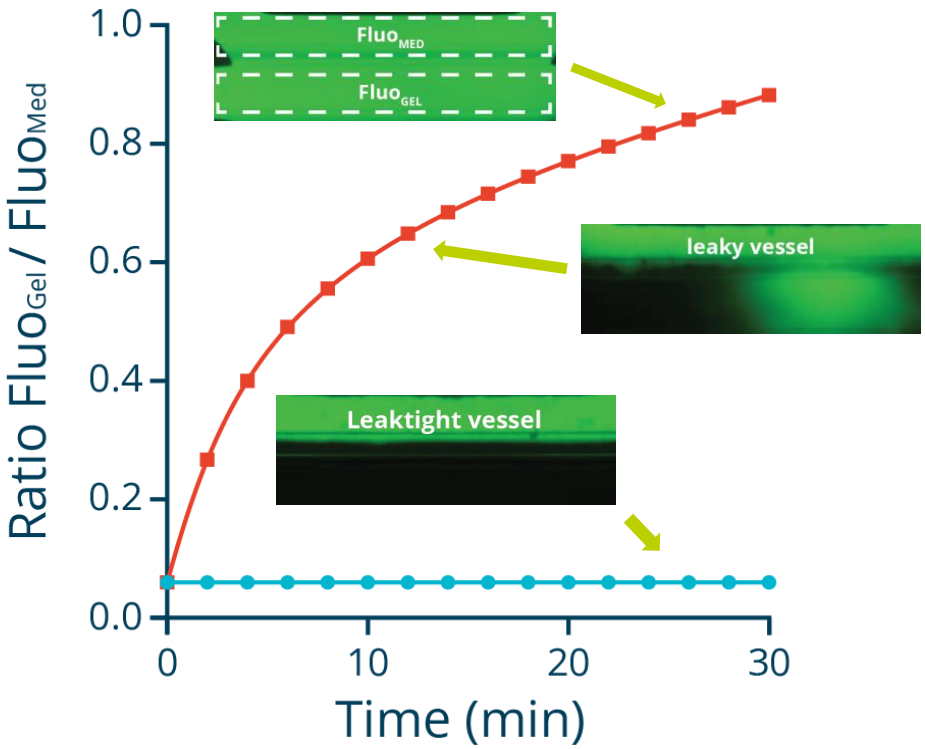


***Figure 2**: Example of quantification of the barrier integrity assay in the OrganoPlate®. In case of a leaktight barrier, the ratio of fluorescent signal measured in the medium channel and the gel channel remains constant and is relatively low (blue line). In case of a leaky barrier or a cell-free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

7. Optional: continue culture

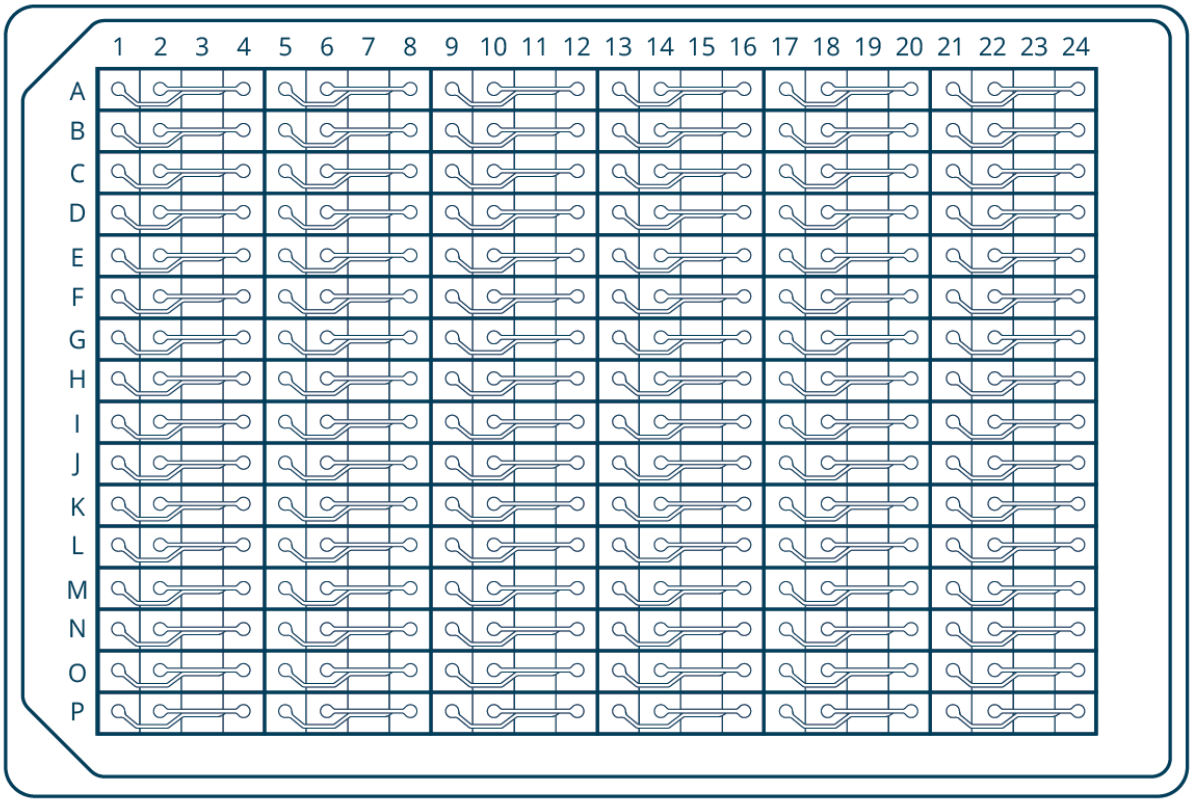
To continue culture of the tubules after performing the barrier integrity assay, aspirate all solutions from the wells and add 50 µL of fresh culture medium to all perfusion inlets and outlets. Then place the OrganoPlate® back on the OrganoFlow® in the incubator to continue culture.

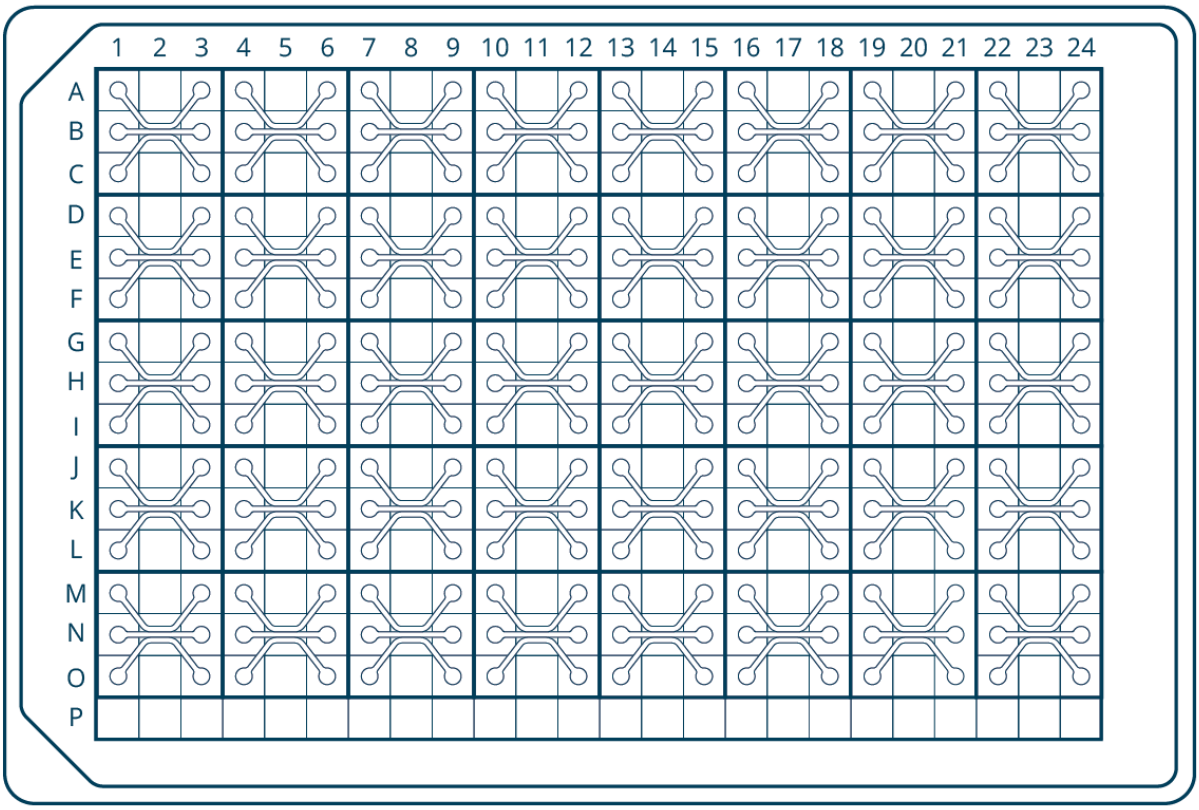
8. Optional: inducing barrier disruption

Barrier disruption can be induced in barrier models grown in the OrganoPlate, e.g. by exposure to toxic compounds or inflammatory triggers. 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

Plates layout

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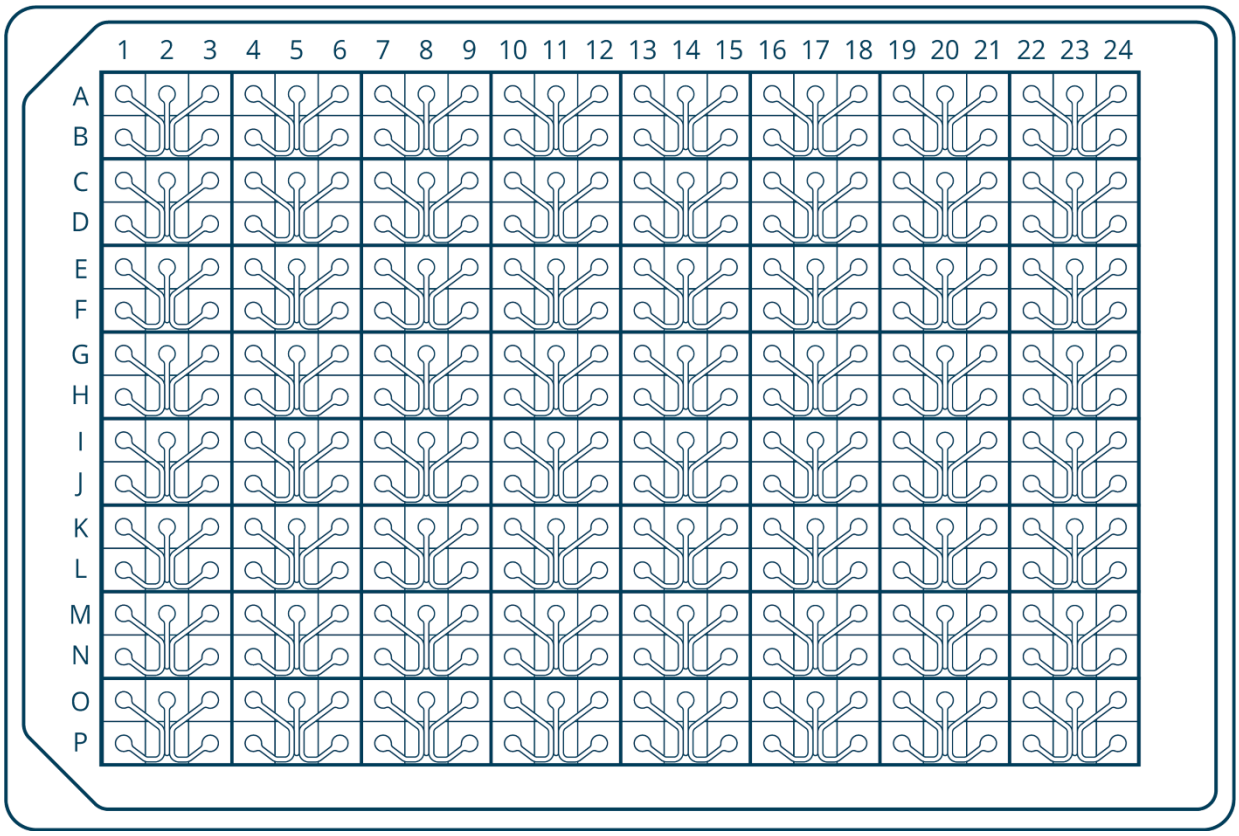
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MIMETAS Product List

Cat. No.	Product Name
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-OR-VB-01	OrganoReady® Vascular Bed HUVEC
MI-OR-CO-CU -01	OrganoReady® Collagen 3-lane 40
MI-OR-CO-CU-02	OrganoReady® Collagen 3-lane 64
9605-400-B	OrganoPlate® 2-lane 96
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-VP2	OrganoTEER® Standard package

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