1. Objective

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

- As one of the criteria for optimizing a tissue model
- To select for leaktight tissues for a transport or toxicant exposure study
- To detect compound-induced disruption of the barrier (end-point 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 leaktight monolayer. The tightness of the monolayer can be assessed with the barrier integrity assay. For this assay, the medium is replaced by medium containing a fluorescent dye and leakage of the dye from the medium 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.

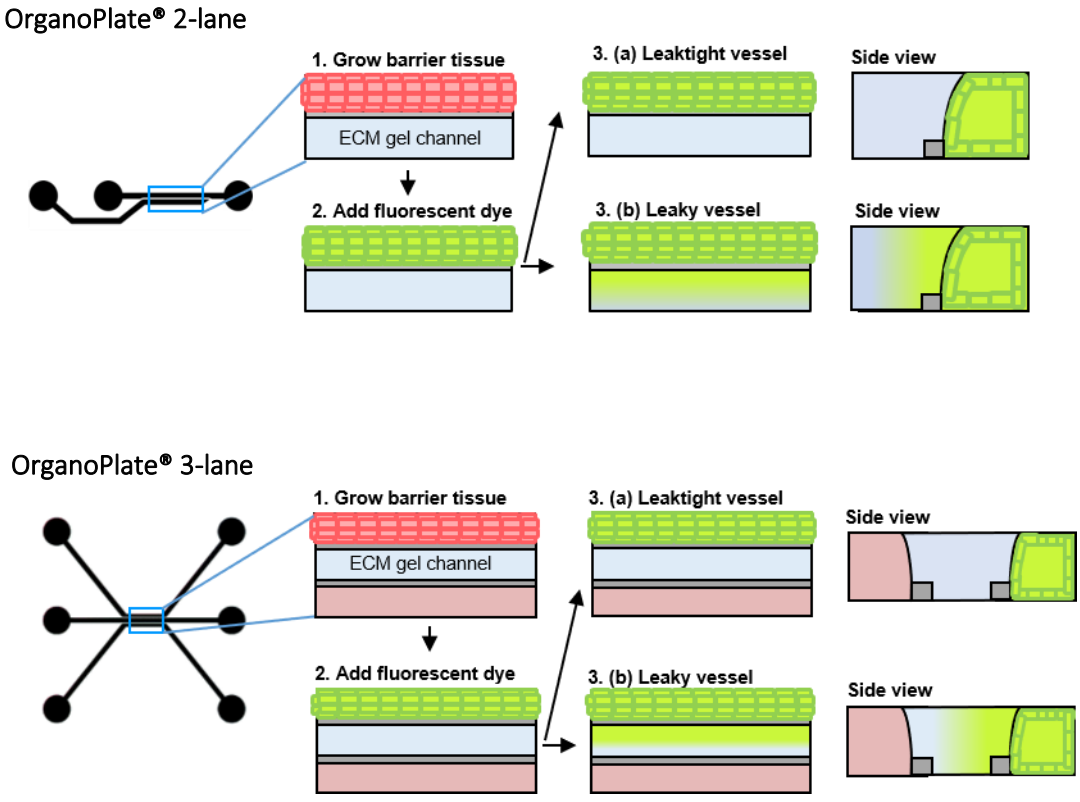


***Figure 1**: Schematic overview of the barrier integrity assay in the OrganoPlate® 2-lane and 3-lane*

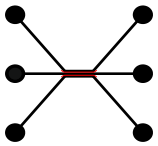
3. Materials

- OrganoPlate® with barrier tissue (i.e. epithelial tubules)
- Medium
- Fluorescent compounds
    a. Commonly used fluorescent compounds 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)
    b. Keep all solutions sterile

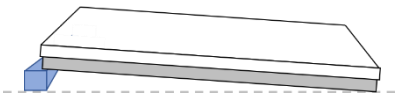
4. Assay

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 compounds
    a. You can assess barrier integrity for several compounds of different sizes in one chip by mixing two differently labeled compounds together in the fluorescent working solution
    b. The final concentration of the fluorescent compounds is 0.5 mg/mL, 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. In case you're working with an OrganoPlate® 3-lane and only the top medium channel (containing the barrier tissue) was perfused during culture and there is no liquid present in the bottom medium 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.
    a) Leave the medium in the top inlet and top outlet
    b) Add 50 µL medium to <u>all</u> 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



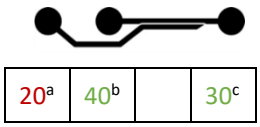
50[a]		50[a]
50[b]		50[b]
50[b]		50[b]



4. In case you are working with an OrganoPlate® 2-lane or an OrganoPlate® 3-lane in which the bottom channel was perfused with medium during culture, step 3 can be omitted. Instead, simply aspirate all medium from all inlets and outlets and proceed to step 5.

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

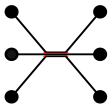
OrganoPlate® 2-lane



20[a]	40[b]		30[c]

a. Pipette 20 µL of regular 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[c]		30[d]
20[a]		20[a]
20[b]		20[b]

a. Pipette 20 µL of regular culture medium in the gel inlet and outlet
b. Pipette 20 µL of regular culture medium in the bottom medium 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

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 medium channel (which represent the lumen) and the adjacent gel channel of a chip, at end-point or over time.
    a. In case the barrier tissue is leaktight, this ratio will remain constant and is relatively low (because the fluorescent signal in the medium 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 medium channel over time).
    c. Figure 2 exemplifies the quantification of the barrier integrity assay

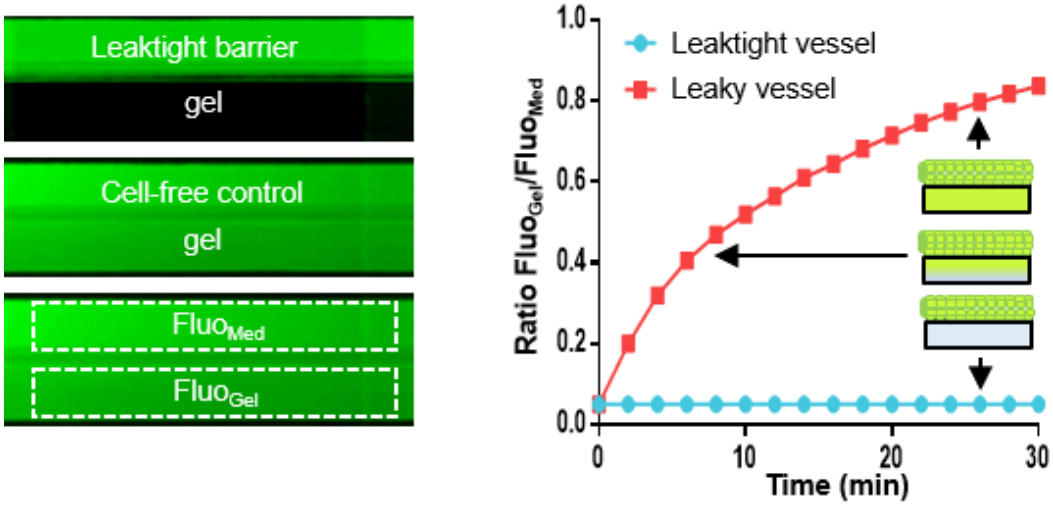


***Figure 2**: Example of quantification of the barrier integrity assay in the 2-lane OrganoPlate®. In case of a leaktight barrier, the ratio of fluorescent signal measured in the medium channel and the gel channel remains constant and is relatively low (blue line). In case of a leaky barrier or a cell-free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

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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