emulate

Protocol for Emulate Organ-Chips:

Barrier Function Analysis

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Goals:	Key Steps:	Other Required Materials:
Analyze effluent from Organ-Chips after running the Barrier Function Assay	• Prepare standard curve • Load standards and samples into the plate • Read plate • Analyze barrier function	• Plate-reader for fluorescent molecules • Emulate Standard Curve Calculator (EC003) • Emulate P_{app} Calculator (EC004)

Introduction

The maintenance or disruption of tissue barriers is an essential part of the pathophysiology of many diseases. The ability to quantitatively characterize tissue barrier is critical in the evaluation of barrier integrity and function.

This protocol is to be used to assess the permeability of an Organ-Chip's endothelial-epithelial barrier. Apparent permeability (P_{app}) of tracer molecules is determined by dosing the inlet of one channel, collecting the effluent of both channels, and calculating the amount of compound that crossed through the membrane over time. See full method below and associated P_{app} Calculator (EC004) for data analysis.

Method

1. Prepare standard curves of the fluorescent tracer molecule used in your permeability assay in both the dosing channel media as well as receiving channel media. This is required to quantify and further analyze barrier integrity on the Organ-Chip.
    1. Start by preparing at least 500 µL of a solution of fluorescent tracer dissolved in the dosing media at the same concentration that is added to the dosing channel reservoir. (You can prepare a larger volume if needed to accommodate greater dilution of the tracer.) Label this solution 1.
       Note: as a starting point, we recommend the following fluorescent tracers and concentrations: Cascade Blue 3kDa (cat #D7132 Thermo Fisher) at 100 µg / mL or Lucifer Yellow (cat #L453 Thermo Fisher) at 20 µg / mL.
    2. Label 7 other tubes 2–8, and add 200 µL of the respective media (dosing or receiving channel media) to each of the tubes.
    3. Take 200 µL solution from tube 1 and add to 2, mixing evenly by pipetting up and down a few times. Then collect 200 µL from 2 and add to 3, continuing this way until you have prepared a serial dilution until tube 7. Add media without compound to tube 8, which will be used as the blank.
    4. Repeat this serial dilution for receiving channel media, except start with a concentration of the fluorescent molecule that is 25% of the concentration added to the donor channel reservoir. (This is done to align the standard curve with the expected recovery concentrations.) Label tubes 9–16.

2. Load a 96-well plate with either 50 or 100 µL (as per the requirements of your plate reader) of each of the solutions from tubes 1–16 (standards), as well as samples collected while running the barrier function assay.
    1. For the most reliable analysis, collect samples from all four reservoirs for every Pod™ Portable Module at each timepoint (i.e., bottom channel inlet reservoir, bottom channel outlet, top channel inlet reservoir, and top channel outlet reservoir as in L-R Pod reservoirs, Figure 1). Inlet concentrations can be used to confirm proper dosing and assess if there is compound loss to the system.
    2. At a minimum, donor and receiver outlet samples must be collected at each time point.
3. Load your plate and read on a plate reader.
4. Using the standard curves generated by the plate reader, quantify the concentration of each samples, making sure to use the curve used for the media type being analyzed (e.g., top or bottom channel).
    1. A linear relationship is expected between concentration and plate reader output — check to ensure that the data is indeed linear in the concentration range being measured from the samples, especially the receiving channels, which is expected to be a much lower concentration than the donor channel.
    2. Perform a linear regression on the standard curve data to determine the equation of the form Y = m*X + b, which is needed to correlate plate reader data to concentrations.
    3. Correlate plate readings to data using the regression equation. The Emulate Standard Curve Calculator (EC003, https://emulatebio.com/protocol-archive/ep187-v1-0) automatically generates the standard curves and converts inputted data based on the curve generated. This calculator performs a log-log linear regression analysis, which minimizes the percent error across the full range of the standard curve data.
5. Once all the concentrations have been calculated, apparent permeability (P_{app}) can be calculated using the following equation, which accounts for any loss of compound by assuming first-order loss dynamics along the length of the chip:

$$P_{app} = -\frac{Q_R * Q_D}{SA * (Q_R + Q_D)} * \ln\left[1 - \frac{C_{R,0} * (Q_R + Q_D)}{(Q_R * C_{R,0} + Q_D * C_{D,0})}\right]$$

where P_{app} is the apparent permeability in units of cm / s; SA is the surface area of sections of the channels that overlap (0.17cm^2); Q_R & Q_D are the fluid flow rates in the dosing and receiving channels respectively, in units of cm^3 / s; and $C_{R,0}$ & $C_{D,0}$ are the recovered concentrations in the dosing and receiving channels respectively, in any consistent units. The Emulate P_{app} Calculator (EC004) automatically performs this calculation. (https://emulatebio.com/protocol-archive/ep187-v1-0.)

Troubleshooting

If you are unable to quantify tracer concentration in the receiving channel because the recovered concentration is below the lower limit of detection of the analytical instrument, consider increasing the dosing concentration of the tracer. This will result in an increase in the recovered concentration and will not affect the calculated P_{app}.

Similarly, if there is difficulty measuring significant tracer concentrations in the receiving effluent, consider decreasing the flow rate of the receiving channel. This will increase the residence time of the tracer in the chip and allow more of the tracer to pass through the membrane, increasing the receiver channel's recovery concentration.

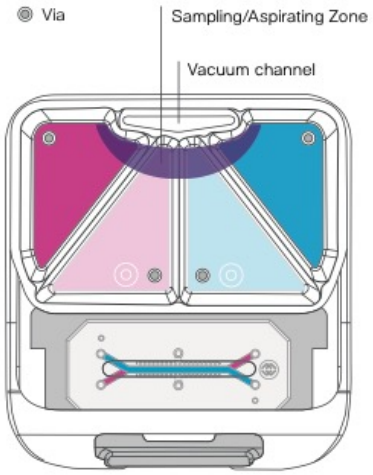

Figure 1