emulate

# Protocol for Emulate Organ-Chips:

# Live Staining of Cells

April 4, 2019

EP155 v1.0

| Goals: | Key Steps: | Other Required Materials: |
|---|---|---|
| **Stain live cells in Emulate Organ-Chips** | • Prepare microscope<br>• Stain live cells | • Diluent for live stain (e.g., cell culture media, PBS)<br>• Conical tubes or Eppendorf Tubes®<br>• Live staining reagent<br>• P0.5–P1000 pipettes and tips<br>• Fluorescence microscope |

## 1. Method

1. Turn on fluorescence microscope prior to live imaging to ensure adequate setup and readiness. Set the live imaging chamber to 37°C and 5% $CO_2$ at least 30 to 60 minutes before starting imaging.
2. Ensure all chip carriers are well labeled; identify the different conditions clearly. Detach chips from Pod™ modules, and organize them in petri dishes.
3. Follow manufacturer's instruction to prepare working solution of live staining reagent.
4. Gently wash each channel once with 200 µL PBS.
5. Place 200 µL tips gently in the outlets of both channels — we recommend using filtered tips for this step. Be careful to not push the tips too hard against the bottom of the chip channel, as this could seal off the outlet and prevent reagents from going through the channel and outlet.
6. Add 100 µL of the live staining working solution to each channel from the inlet, leaving the tips inserted into the inlet ports as shown in Figure 1 below.

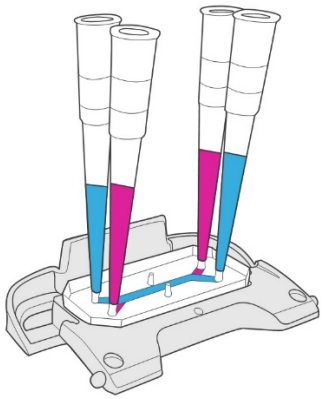

Figure 1 Pipette tips inserted in Chip-S1™ ports

7. Incubate chips according to manufacturer's instructions for live staining reagent.
8. After incubation, remove all four pipette tips and wash each channel with 200 µL of diluent.
9. Image chip immediately, or process further steps following manufacturer's instruction.

   Note: Some live stained signals are retained for certain duration after fixation.