emulate

Duodenum Intestine-Chip Culture Protocol

January 24, 2020

EP184 v1.0

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Introduction to the Human Emulation System®

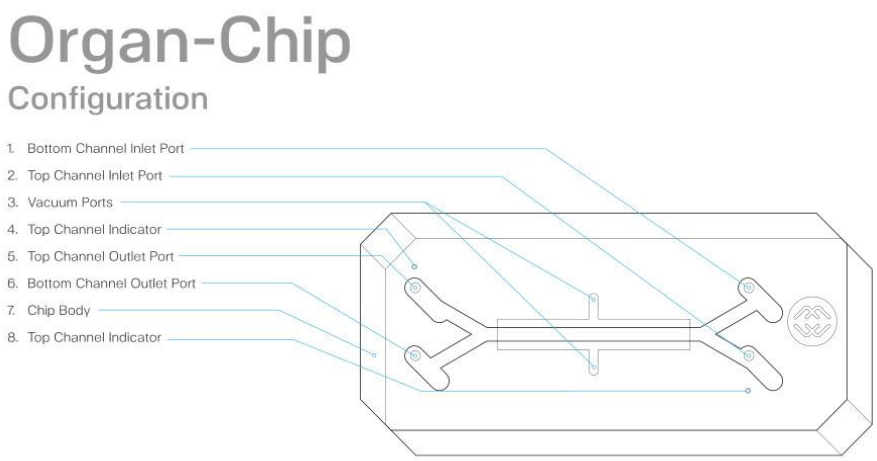
This section provides an overview of key components of the Human Emulation System®, including the Chip-S1™, the Pod™ Portable Module, the Zoë® Culture Module, and the Orb™ Hub Module.

An Organ-Chip is a living, micro-engineered environment that recreates the natural physiology and mechanical forces that cells experience within the human body. The Pod provides the media to the Organ-Chip that is needed to promote cell growth; the Pod also acts as the interface between the Organ-Chip and the Zoë Culture Module. Zoë provides the flow of nutrient-rich media at a rate determined by the user and provides the mechanical forces that emulate the physical forces experienced *in vivo* by tissue. The Orb delivers a mix of 5% CO_2, vacuum stretch, and power for up to four Zoës.

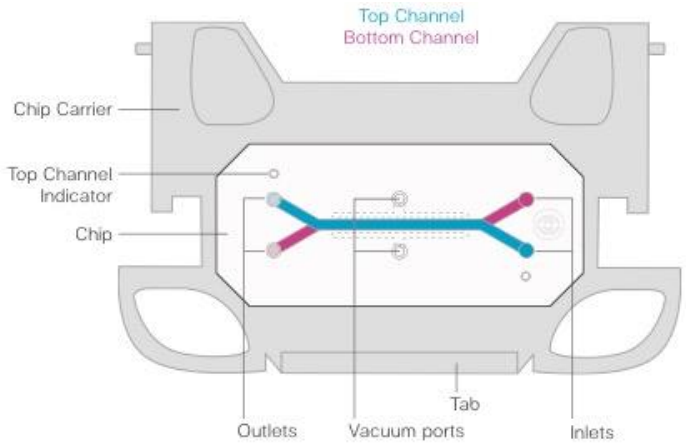
Organ-Chip (Chip-S1™)

Our Chip-S1™ can be configured to emulate multiple different organ tissues including lung, liver, intestine, kidney, and brain. The chip, when connected to the Pod and Zoë, recreates the body's dynamic cellular microenvironment, including tissue-to-tissue interfaces, blood flow, and mechanical forces. This protocol describes the methodology for the Duodenum Intestine-Chip.

The microenvironment created within each Chip-S1 includes epithelial cells in the top channel and endothelial cells in the bottom channel. Top and bottom channels are separated by a porous membrane that allows for cell-cell interaction like those that are seen *in vivo*. These two channels are fluidically independent. Cells in each channel are seeded with organ- and cell-specific extracellular matrix proteins (ECM), and can be maintained in static culture for up to four days, depending on cell type, before being connected to Zoë, which provides continuous flow of cell culture media.



Chips are supplied pre-packaged in the chip carrier and should be maintained in the chip carrier at all times. The chip carrier is designed to hold chips in place, eliminating the need to disturb or distort the chip during preparation. In addition, the chip carrier connects securely to the Pod, which acts as the interface between Zoë and the chip. Use sterile tweezers when handling chips in chip carriers. Removal of the chip from the chip carrier prior to or during an experiment could result in chip failure. (The only appropriate time to remove the chip from the carrier is at end of the life of the chip in the experiment, when it is no longer used for live culture of the cells.)



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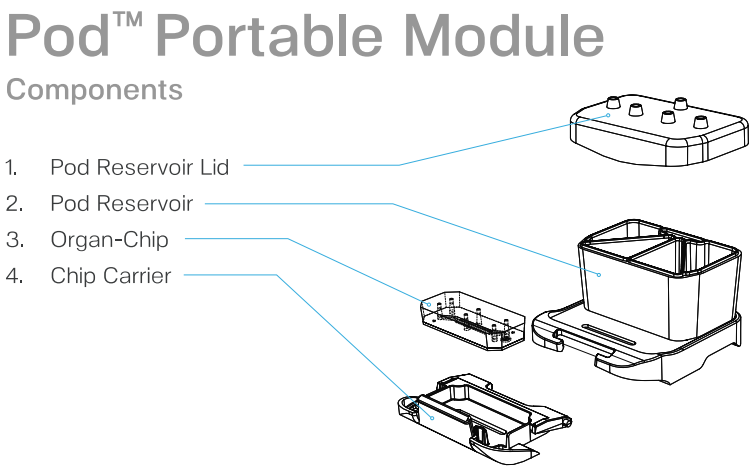
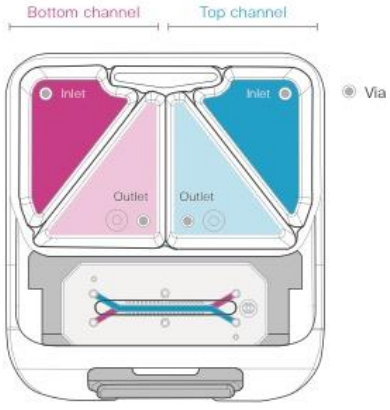
Pod™ Portable Module

The Pod™ Portable Module houses the chip, supplies media, and enables compatibility with microscopes and other analysis equipment. The Pod, which has a height of 41.5 mm, is designed to allow Organ-Chips to be easily transported and placed on standard microscopes for imaging. It also allows simple portability of Organ-Chips between our hardware and other analysis equipment.

The Pod's reservoirs allow users to introduce nutrient media or blood, precisely control dosing to test drugs or other inputs, and sample chip effluent. Each Pod reservoir holds up to 4 mL.





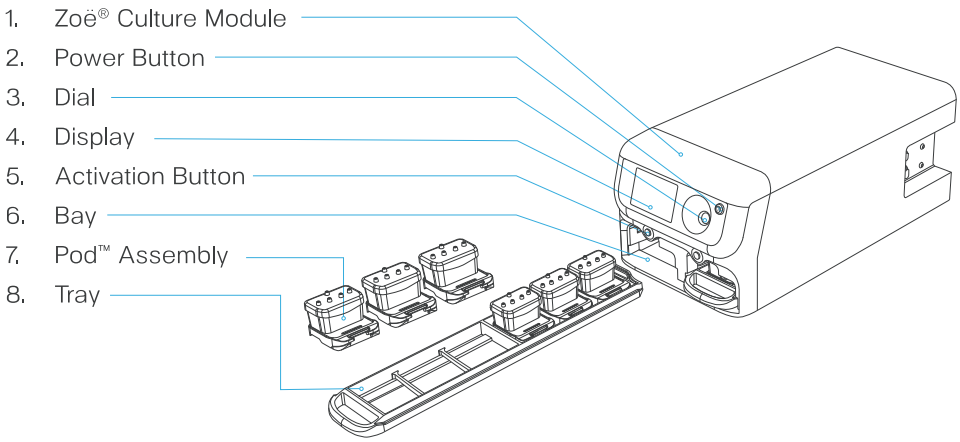
Zoë® Culture Module

The Zoë® Culture Module is designed to sustain the life of cells within Emulate's Organ-Chips. It provides the dynamic flow of media and the mechanical forces that help recreate the microenvironment cells experience *in vivo*. The instrument automates the precise conditions needed for simultaneous cell culture of up to 12 Organ-Chips.

Zoë enables independent control of the flow rate of media through the top and bottom channels of the Organ-Chips, as well as stretch parameters — including frequency and amplitude — of the Organ-Chip. Additionally, Zoë has automated algorithms to prime the fluidic channels of Pods with media, and programming to maintain the culture microenvironment for optimal cell performance.

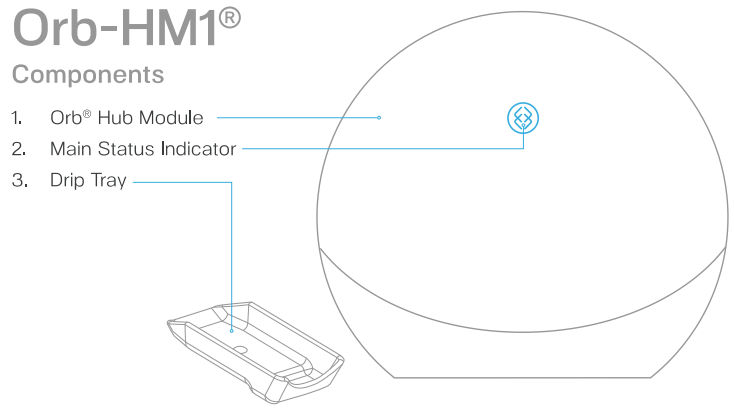
Zoë-CM1®

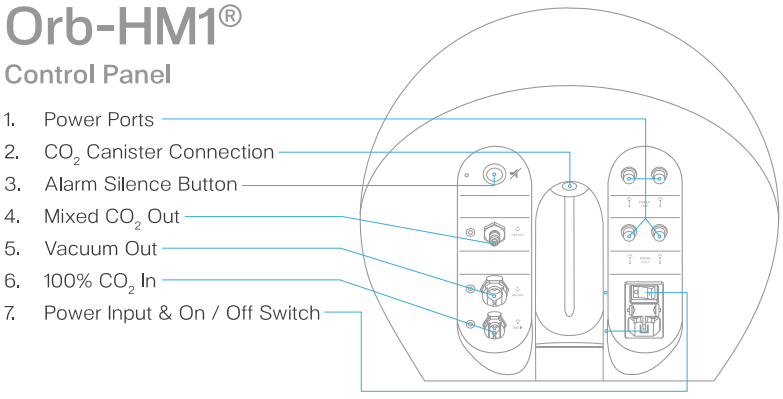
Components



Orb™ Hub Module

The Orb™ Hub Module is a central hub that connects to up to four Zoë® Culture Modules. It provides a simple solution for installing and operating the Human Emulation System within the lab environment. The Orb delivers a mix of 5% CO_2, vacuum stretch, and power from standard lab connections. It generates a 5% CO_2 supply gas to Zoë by combining air with an external 100% carbon dioxide supply (or from a portable CO_2 canister for increased flexibility) in a controlled mixture. Orb also generates the vacuum required to apply Organ-Chip stretching (-70 kPa), and contains four individual power ports.





Cells

The commercial availability of human duodenal organoids is limited. We recommend testing various sources and donors of cells for viability, morphology, and expression of key markers associated with fully differentiated duodenal intestinal epithelium.

We recommend users to use healthy organoids generated from isolated duodenal intestinal crypts.

For the endothelial cells, we recommend Human intestinal microvascular endothelial cells isolated from small intestine (siHIMECs) from AlphaBioregen (#ALHE15)

Media and Gas Equilibration

Proper gas equilibration of media is essential for successful Organ-Chip culture. As the system is sensitive to bubbles, all media must be equilibrated to 37°C and excess gas must be removed prior to use in the chip and in Zoë. To do this, 37°C medium is placed under vacuum using the 0.45 µm PVDF filter of a Steriflip® conical filter unit. Failure to carry out this step could lead to failure of the chips in the experiment.

It is also important to look for bubbles throughout the experiment. Observed bubbles can often be flushed out of the system to avoid a disruption in medium flow and / or improper surface coverage. However, instances of chips with visible bubbles or blocked flow should be recorded and considered when analyzing experimental data.

Experimental Overview

Workflow Overview

Day X: Reagent Preparation

- Aliquot reagents (media supplements, ECM, Matrigel, etc.)

Day -1: Chip Preparation

- Prepare chips
- Prepare ER-1 solution
- Introduce ER-1 solution to channels
- Activate and wash chips
- Prepare ECM solutions
- Coat chips with ECM solutions

Day 0: Organoids to Chips

- Prepare Organoid Medium
- Prepare Dissociation Solution
- Prepare Chips
- Recover organoids from Matrigel
- Adjust cell density
- Seed fragmented organoids to top channel

Day 1: Chips to Pods and Pods to Zoë

- Gas equilibration of media
- Prime Pods
- Wash chips
- Chips to Pods
- Pods to Zoë

Day 2: Maintain Chips in Zoë; Thaw Human Intestinal Microvascular Endothelial Cells (HIMECs)

- Via wash Pods
- Prepare HIMEC culture medium
- Prepare T75 flask
- Thaw and plate HIMECS

Day 3: Replace Media in Chips and HIMEC Culture

- Prepare organoid maintenance medium
- Sampling and media replenishment
- Maintain HIMECs in culture

Day 6: Seed HIMECs to Chip; Connect Chips to Pods and Pods to Zoë; Add 2% Stretch

- Prepare counting solution

- Prepare chips
- Harvest HIMECs
- Seed HIMECs to bottom channel
- Gravity wash chips
- Gas equilibration of media
- Prime Pods
- Chips to Pods
- Pods to Zoë
- Add 2% stretch

Day 7: Maintain Chips in Zoë; Increase to 10% Stretch

- Via wash Pods
- Increase stretch to 10%, 0.2 Hz

Day 8+: Maintenance and Sampling

- Sampling and Media Replenishment

Required Materials

Ensure all equipment, materials, and reagents are accessible prior to beginning each experiment. Exact catalog numbers are provided for specific materials required for successful experiments.

Equipment & Materials

Equipment	Description	Supplier	Catalog Number
Zoë CM-1® Culture Module	1 per 12 chips	Emulate	
Orb-HM1® Hub Module	1 per 4 Zoës	Emulate	
Chip-S1™	12 per Zoë	Emulate	
Pod™ Portable Modules	1 per Chip-S1	Emulate	
UV Light Box	1 per Zoë	Emulate	
Chip Cradle	Autoclaved, 1 per 6 chips	Emulate	
Steriflip®-HV Filters	0.45 µm PVDF filter sterile	EMD Millipore	SE1M003M00
150 mm cell culture dish	Sterile, 1 per 6 chips	Corning / Falcon	353025

Costar® 24-well Clear TC-treated Multiple Well Plates, Individually Wrapped, Sterile	24 well, flat-bottom TC-treated	Corning	3524
Handheld vacuum aspirator		Corning	4930
Aspirating pipettes	2 mL, polystyrene, individually wrapped	Corning / Falcon	357558
Aspirating tips	Sterile (autoclaved)		
Serological pipettes	2 mL, 5 mL, 10 mL, and 25 mL low endotoxin, sterile		
Pipette	P20, P200, and P1000		
Pipette tips	P20, P200, and P1000 sterile, low-adhesion		
Conical tubes	15 mL and 50 mL polypropylene, sterile		
Eppendorf Tubes®	1.5 mL, sterile		
Mini Cell Scrapers	200 items, sterile	Biotium	22003
Aluminum foil			
Parafilm®			
Microscope (with camera)	For bright-field imaging		
Hemocytometer			
Manual counter			
Water bath (or beads)	Set to 37°C		
Vacuum set-up	Minimum pressure: -70 kPa		
T75 flasks			
Ice bucket			
70% ethanol and wipes	For surface sterilization		

Reagents, Media, & Supplements

Reagent	Description	Supplier	Catalog Number
ER-1	5 mg powder	Emulate	ER105
ER-2	25 mL bottle	Emulate	ER225
Dulbecco's PBS (DPBS -/-) (without Ca^{2+}, Mg^{2+})	1X	Corning	21-031-CV
Trypan blue	0.4% solution	Sigma	93595
TrypLE Express	1X, no phenol red	ThermoFisher Scientific	12604013
IntestiCult™ Human Organoid Growth Medium Kit	Component A and Component B	Stemcell Technologies	06010
Endothelial Cell Growth Medium MV2 Kit	Kit containing basal medium and supplements	Promocell	C-22121
Fetal bovine serum (FBS)	Sterile, heat-inactivated	Sigma	F4135 or F8317
Primocin™	50 mg / mL	InvivoGen	ANT-PM-1
Attachment Factor™	1X	Cell Systems	4Z0-210
Matrigel - Growth Factor Reduced	Phenol Red Free	Corning	356231
Collagen IV	Lyophilized Powder	Sigma	C5533
Fibronectin	Lyophilized powder	Corning	356008
Y-27632	ROCK inhibitor; lyophilized powder	Stemcell Technologies	72302
CHIR99021	Lyophilized powder	Reprocell	04-0004-10
Cell Recovery Solution	Recovery solution	Corning	354253
Advanced DMEM/F12	Media	ThermoFisher	12634028
BSA	30%, Sterile	Sigma	A9576
Cell Culture Grade Water	Sterile, Water	Corning	MT25055CV
DMSO	Sterile	Sigma	D2650

Notes for ER-1 and ER-2

- Upon arrival, store ER-1 (powder) unopened in the metalized pouch at -20°C, protected from light and humidity.
- Upon arrival, store ER-2 solution at 4°C.

Aseptic Technique

- Prepare and sanitize the work surface of the biosafety cabinet (BSC) prior to beginning. Ensure that the work space within the BSC is organized and free from clutter. Arrange tips, media, and other necessary materials in the sterile field, easily within reach, without blocking the path of airflow.
- Sanitize all reagents and materials entering the BSC with 70% ethanol. Spray and wipe thoroughly.
- Always avoid touching the chip directly.
- Handle the chip carrier only by the sides or by the tab with gloves.
- Never remove the chip from the chip carrier prior to performing an experiment.

Cell Storage

Cryopreserved cells must be stored in liquid nitrogen. Severe damage to the cell membrane and cytoskeletal components can result from chronic temperature fluctuations. Never store in dry ice or a -80°C freezer.

Chip Handling Techniques

Always work with chips within the chip carrier in a sterile environment, such as the BSC.

When pipetting to fill each channel, 50 µL volume is generally used for the top channel, and 20 µL is used for the bottom channel. These volumes allow for simple pipetting and a slight overfill to avoid bubbles or dry channels. All wash steps, unless otherwise stated, are performed using 200 µL of the specific wash solution.

While 50 µL (top channel) and 20 µL (bottom channel) are standard volumes used throughout the protocol, there can be some flexibility in the actual volumes used.

Top Channel: 35–50 µL

Bottom Channel: 15–20 µL

The specific channel and membrane dimensions are outlined below:

Top Channel	
Width x height dimensions	1000 µm x 1000 µm
Area	28.0 mm^2
Volume	28.041 µL
Imaging distance from bottom of chip to top of membrane	850 µm
Bottom Channel	
Width x height dimensions	1000 µm x 200 µm
Area	24.5 mm^2
Volume	5.6 µL
Membrane	
Pore diameter	7.0 µm
Pore spacing	40 µm (hexagonally packed)
Thickness	50 µm
Co-Culture Region	
Area	17.1 mm^2

A P200 pipette with a sterile pipette tip is used to add solution directly to the channels of the chip, as when coating, washing, and seeding cells prior to attaching the chip to Zoë. To introduce solution to the channels, place the pipette tip perpendicular to chip channel inlet, ensuring that the tip is securely in the port, and steadily dispense liquid through the channel. Always introduce liquid to the bottom channel before pipetting into the top channel.

Duodenum Intestine-Chip Culture Protocol

Day X: Reagent Preparation

Aliquot Reagents

Aliquot reagents including media supplements and ECM prior to use and store at -20°C to avoid multiple freeze-thaw cycles.

1. Y-27632 (ROCK inhibitor)

Reagent	Conc. [Stock]	Amount	Volume	Solvent
Y-27632	10 mM	5 mg	1.56 mL	0.1% BSA in DPBS

- Resuspend 5 mg of Y-27632 compound in 1.56 mL of 0.1% BSA in DPBS according to manufacturer's instructions. yielding a stock concentration of 10 mM.
- The final concentration of Y-27632 used in Organoid Growth Medium will be 10 µM.
- Aliquot reconstituted Y-27632 to single-use volumes and store at -20°C.

2. CHIR99021

Reagent	Conc. [Stock]	Amount	Volume	Solvent
CHIR99021	5 mM	10 mg	4.29 mL	DMSO

- Resuspend 10 mg of CHIR99021 compound in 4.29 mL of DMSO according to manufacturer's instructions, yielding a stock concentration of 5 mM.
- The final concentration of CHIR99021 used in Organoid Growth Medium will be 5 µM.
- Aliquot to single-use volumes and store at -20°C.

3. Matrigel – Growth Factor Reduced (GFR)

Reagent	Volume [Stock]
Matrigel - Growth Factor Reduced	10 mL

- The stock bottle of Matrigel must be thawed overnight on slushy ice in a 2°C to 6°C refrigerator (in the back) or cold room and handled on slushy ice at all times. (Maintain Matrigel on ice at all times, as this solution gels rapidly at temperatures above 4°C.)
- After thawing, aliquot Matrigel to suitable single-use volumes based on the specific stock concentration and amount needed in the experiment.
    - Keep all materials on ice at all times.
    - Use pre-chilled pipettes, tips, and tubes at -20°C prior to aliquoting.
    - Freeze aliquots immediately at -20°C.
- Thaw aliquots on ice just prior to use.
- Once aliquots are thawed, do not re-freeze.

4. Collagen IV

Reagent	Conc. [Stock]	Amount	Volume	Solvent
Collagen IV	1 mg/mL	5 mg	5 mL	Cell Culture Grade Water

5. Fibronectin

Reagent	Conc. [Stock]	Amount	Volume	Solvent
Fibronectin	1 mg/mL	5 mg	5 mL	Cell Culture Grade Water

Day -1: Chip Preparation

Goals:

- Activate the inner surface of the chip channels for proper ECM attachment
- Coat top channels with an ECM mixture of collagen IV and Matrigel
- Coat bottom channels with an ECM mixture of Collagen IV and fibronectin

Key Steps:

- Prepare Chips
- Prepare ER-1 Solution
- Introduce ER-1 Solution to Channels
- Activate and Wash Chips
- Prepare ECM Solutions
- Coat Chips with ECM Solutions

Required Materials:

- Chip-S1™ (12 chips per Zoë)
- ER-1 powder
- ER-2 solution
- 15 mL conical tubes
- DPBS (-/-) at room temperature
- DPBS (-/-) aliquot at 4°C
- Collagen IV
- Matrigel
- Fibronectin
- 70% ethanol
- 150 mm cell culture dish
- Ice and ice bucket
- Pipettes and filtered tips
- Aspirator and sterile tips
- Aluminum foil
- UV light box
- UV safety glasses

Prepare Chips

1. Spray the packaging of the chips with 70% ethanol and bring into the BSC.
2. Open the packaging carefully, and lay out 6 chips in each sterile 150 mm dish. Note: For ease of workflow, align all chips facing the same direction in the 150 mm dish. To properly orient the chips, ensure the tab of the carrier is facing to the right. (See Figure 1)
3. Label each chip with ID numbers on the chip carrier tab.

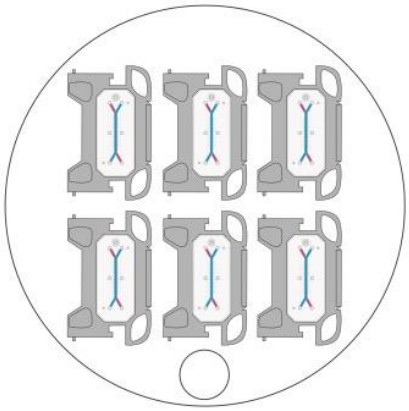


Figure 1: Proper orientation of Organ-Chips in 150 mm dish

Prepare ER-1 Solution

ER-1 is light-sensitive. Prepare ER-1 solution immediately before use and discard any remaining ER-1 solution 1 hour after reconstitution. Failure to protect from light or use of ER-1 solution that has not been freshly prepared will lead to failure of chips. Note: ER-1 is an eye irritant and must be handled in the BSC with proper gloves and eye protection.

1. Turn off the light in the BSC and allow ER-1 and ER-2 to equilibrate to room temperature before use (approximately 10 to 15 minutes).
2. Wrap an empty, sterile 15 mL conical tube with foil to protect it from light.
3. In the BSC, remove the small vial of ER-1 powder from the packet, and briefly tap the vial to concentrate the powder at the bottom.
4. Add 1 mL of ER-2 to the vial, and transfer contents directly to the bottom of the 15 mL conical tube. Do not pipette to mix. Note: The color of the solution transferred to the conical tube will be deep red.
5. Add an additional 1 mL of ER-2 to the ER-1 vial to collect any remaining material, and transfer the solution directly to the 15 mL conical tube. Note: The color of the transferred ER-1 solution will become lighter with each additional wash of the ER-1 bottle.

6. Repeat Step 5 twice more, with an additional 1 mL of ER-2 each time.
7. On the last addition of 1 mL ER-2 to the ER-1 bottle, cap the bottle and invert to collect any remaining ER-1 powder from the lid. Transfer the collected solution to the conical tube, bringing the total volume in the tube to 4 mL ER-1 solution.
8. Add 6 mL of ER-2 solution to the 4 mL of ER-1 solution in the 15 mL conical tube (for a final working concentration of 0.5 mg / mL). Pipette gently to mix without creating bubbles. ER-1 should be fully dissolved within the ER-2 solution prior to use.

Introduce ER-1 Solution to Channels

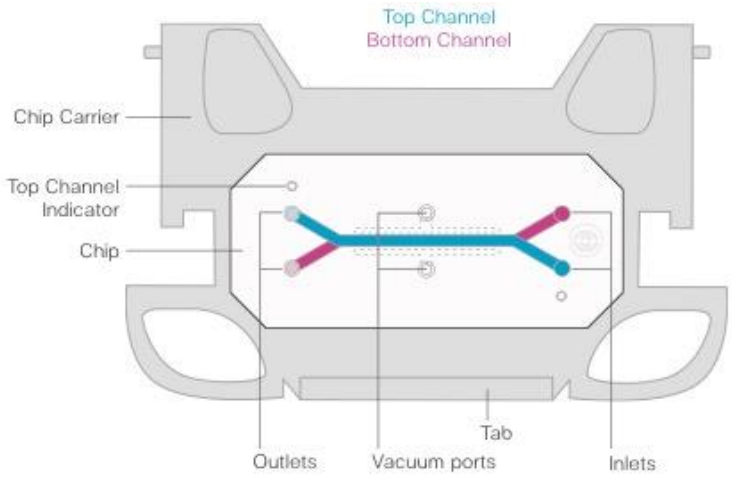


Figure 2: Top view of chip, with labelled ports, in chip carrier

1. Using a P200 pipette and a sterile 200 µL filtered pipette tip, take up 200 µL of ER-1 solution. Note: 200 µL of ER-1 solution will fill approximately 3 chips.
2. Carefully introduce approximately 20 µL of ER-1 solution through the bottom channel inlet, pipetting until the solution begins to exit the bottom channel outlet (see Fig. 2 above).
3. Without releasing the pipetting plunger, take the pipette out from the bottom channel inlet, and move the pipette containing the remaining ER-1 solution to the top channel inlet.
4. Introduce approximately 50 µL of ER-1 solution to the top channel inlet, pipetting until the solution begins to exit the top channel outlet (see Fig. 2 above).
5. Remove all excess ER-1 solution from the surface of the chip by gentle aspiration. Be sure to only remove ER-1 solution from the chip surface — do not aspirate ER-1 from the channels. (See Figure. 3 below.)
6. Repeat Steps 1 through 5 for each chip.
7. Inspect the channels for bubbles prior to UV activation. If bubbles are present, dislodge by washing the channel with ER-1 solution until all bubbles have been removed. If bubbles persist, it may be helpful to aspirate the channel dry and slowly re-introduce ER-1 solution.

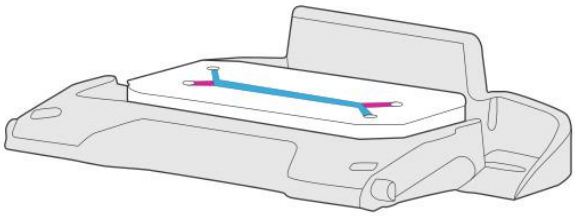


Figure 3: View of chip with no excess ER-1 solution on surface

Activate and Wash Chips

1. Bring the 150 mm dish containing the ER-1-coated chips to the UV light box.
2. Remove the cover from the 150 mL dish and place the open dish in the UV light box.
3. Set the switch at the back of the UV light box to the “Constant” setting. Turn on the power, and press the on button to begin UV activation.
4. Allow the chips to activate under UV light for 20 minutes.
5. While the chips are being treated, prepare ECM Solution. (For more information, see the next section: Prepare ECM Solution.)
6. After UV treatment, bring chips back to the BSC. Note: The light may be on in the BSC from this point forward.
7. Fully aspirate the ER-1 solution from both channels.
8. Wash each channel with 200 µL of ER-2 solution.
9. Fully aspirate the ER-2 from the channels.
10. Wash each channel with 200 µL of sterile cold DPBS.
11. Leave cold DPBS inside the channels.

Prepare ECM Solutions

The ECM solution is prepared fresh each time by combining the individual ECM components with cold DPBS to the final working concentrations. Separate ECM solutions will be used to coat the top and bottom channels.

For human Duodenum Intestine-Chip, the final concentration of proteins in the ECM is:

Top channel: Collagen IV: 200 µg / mL

Matrigel: 100 µg / mL

Bottom channel: Collagen IV: 200 µg / mL

Fibronectin: 30 µg / mL

1. Bring an ice bucket and ice to the BSC.
2. Thaw one aliquot of fibronectin (1 mg / mL), Collagen IV (1mg/ml) and Matrigel (concentration is dependent on specific lot used) on ice. Maintain all ECM components and mixture on ice at all times.
3. Calculate total volume of ECM solution needed to coat all chips.
    1. Volume required per channel = approximately 100 µL
    2. For every 12 chips to coat, prepare 1.5 mL of each ECM solution (12 chips x 100 µL / chip + extra 300 µL = 1.5 mL of each ECM solution). (See calculation example below.)
4. Combine components to prepare ECM working solutions.
5. Keep the ECM solutions on ice until ready to use.

Calculation Example – Top Channel

Collagen IV [stock] = 1 mg / mL (C_1)

Collagen IV [final] = 0.2 mg / mL (C_2)

Matrigel [stock] = 10 mg / mL (C_1)

Matrigel [final] = 0.1 mg / mL (C_2)

Total volume of ECM solution = 1.5 mL (V_2)

Collagen IV

$C_1V_1 = C_2V_2$ → solve for V_1

(1 mg / mL) x (X mL) = (0.2 mg / mL) x (1.5 mL)

X = 0.3 mL = 300 µL of collagen IV stock solution

Matrigel

$C_1V_1 = C_2V_2$ → solve for V_1

(10 mg / mL) x (Y mL) = (0.1 mg / mL) x (1.5 mL)

Y = 0.015 mL = 15 µL of Matrigel stock solution

DPBS

DPBS = (total volume of ECM needed) – (volume of collagen IV) – (volume of Matrigel)

= 1500 µL – 300 µL – 15 µL

= 1,185 µL of DPBS

Calculation Example – Bottom Channel

Collagen IV [stock] = 1 mg / mL (C_1)

Collagen IV [final] = 0.2 mg / mL (C_2)

Fibronectin [stock] = 1 mg / mL (C_1)

Fibronectin [final] = 0.03 mg / mL (C_2)

Total volume of ECM solution: 1.5 mL (V_2)

Collagen IV

$C_1V_1 = C_2V_2$ → solve for V_1

(1 mg / mL) x (X mL) = (0.2 mg / mL) x (1.5 mL)

X = 0.3 mL = 300 µL of collagen I stock solution

Fibronectin

$C_1V_1 = C_2V_2$ → solve for V_1

(1 mg / mL) x (Y mL) = (0.03 mg / mL) x (1.5 mL)

Y = 0.045 mL = 45 µL of Fibronectin

DPBS

DPBS = (total volume of ECM needed) – (volume of collagen IV) – (volume of fibronectin)

= 1500 µL – 300 µL – 45 µL

= 1,155 µL of DPBS

Coat Chips with ECM Solutions

1. Fully aspirate the cold DPBS from both channels.
2. Set a P200 pipette to take up 100 µL of bottom channel ECM solution. (100 µL of ECM solution will be used per chip per channel.)
3. Carefully introduce the bottom channel ECM solution through the bottom channel inlet until a small ECM droplet forms on the bottom channel outlet.
4. Pipet the remaining ECM in the tip as a droplet over the bottom channel inlet. (See Figure 3.)
5. Repeat steps 3 and 4 to coat the bottom channels of each chip.
6. Carefully introduce the top channel ECM solution through the top channel inlet until a small ECM droplet forms on the top channel outlet.
7. Pipet the remaining ECM in the tip as a droplet over the top channel inlet.

8. Repeat steps 6 and 7 to coat the top channel of each chip.
9. Inspect channels to ensure that no bubbles are present. If bubbles are present, dislodge by washing the channel with appropriate ECM solution until all bubbles have been removed.
10. Add 1.5 mL of DPBS to the cap of a 15 mL conical tube. Place the PBS cap in the 150 mm culture dish with the chips to provide extra humidity and seal the dish.
11. Parafilm the dish and incubate the chips in the incubator at 37°C overnight.

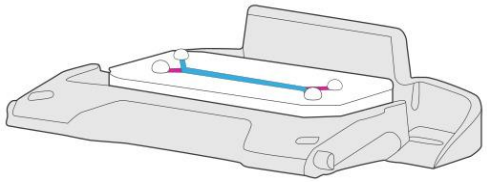


Figure 3: Chip in chip carrier with small droplets of ECM solution at ports

Day 0: Organoids to Chips

Goals:

- Seed organoids in the top channel of chip

Key Steps:

- Prepare Organoid Medium
- Prepare Dissociation Solution
- Prepare Chips
- Recover organoids from Matrigel
- Adjust cell density
- Seed fragmented organoids to top channel

Required Materials:

- Complete Organoid Medium
- 15 mL conical tube
- Cell Recovery Solution
- Dissociation Solution
- TrypLE
- Y-27632
- CHIR99021
- Advanced DMEM/F12
- Mini cell scraper
- Serological pipettes
- Pipettes and tips
- Aspirator
- Centrifuge (set to 4°C)
- Ice bucket, ice
- 70% ethanol

Prepare Organoid Medium

Reagent	Volume	Conc. [Stock]	Conc. [Final]	Source	Cat. No.
IntestiCult™ OGM Component A	50 mL	-	-	Stemcell Technologies	06010 (kit); 06011 (part)
IntestiCult™ OGM Component B	50 mL	-	-	Stemcell Technologies	06010 kit; 06012 (part)
Primocin	200 µL	50 mg / mL	100 µg / mL	InvivoGen	ANT-PM-1
Y-27632	100 µL	10 mM	10 µM	Stemcell Technologies	72302
CHIR99021	100 µL	5 mM	5 µM	Reprocell	04-0004-10

- IntestiCult™ Organoid Growth Medium (Human) (06010) is supplied as a kit containing IntestiCult™ OGM Human Component A (06011) and IntestiCult™ OGM Human Component B (06012).
- Primocin is a non-toxic antimicrobial agent for primary cells.
- Y-27632 is a selective inhibitor of ROCK1 and ROCK2 used to enhance survival of cells.
- CHIR99021 is a small molecule inhibitor glycogen synthase kinase 3 β (GSK-3β).
- Store prepared Complete Organoid Growth Medium at 4°C for up to 7 days.

Prepare Dissociation Solution

Reagent	Volume	Source	Cat. No.
TrypLE	5 mL	GIBCO	12604013
DPBS	5 mL	Sigma	D8537
Y-27632	10 µL	Sigma	72302

- Prepare Dissociation Solution fresh for each use.

Prepare Chips

1. Transfer the ECM-coated chips to the BSC.
2. Fully aspirate ECM from both channels of each chip.
3. Gently wash each channel with 200 µL of Complete Organoid Growth Medium, aspirating the medium outflow, but leaving a small droplet of medium on the surface of the chip.
4. Cover the 150 mm dish and place the chips in the incubator until the organoids are ready for seeding.

Recover Organoids from Matrigel

Prior to recovering organoids, ensure all equipment and materials are ready to use, organized, and that all required reagents are prepared and are at the appropriate temperature.

1. Transfer the 24 well plate containing organoids into the BSC.
2. Carefully aspirate medium from each well without disturbing the Matrigel dome.
3. Gently add 500 uL of Cell Recovery Solution to each well.
4. Scrape the Matrigel dome using a cell scraper.
5. Using a P1000 pipette, collect contents of each well and transfer to a cold 15 mL conical tube.
6. Incubate conical tube on ice for 45 minutes, inverting the tube every 2-5 minutes during this time.
7. While cells are incubating on ice, ensure the centrifuge is cooled to 4°C.
8. Centrifuge the organoid suspension at 300 x g for 5 minutes at 4°C.
9. After centrifugation, observe the tube to confirm complete disappearance of Matrigel and formation of a clear cell pellet.
   Note: If a thin layer of Matrigel is present, or no clear pellet is formed, aspirate the supernatant without disrupting the Matrigel layer, and add 5 mL of new Cell Recovery Solution. Incubate for 5 minutes on ice, and repeat centrifugation in step 8.
10. Once a defined cell pellet is observed, aspirate the supernatant and add 2 mL of prepared Dissociation Solution.
11. Mix gently to re-suspend the pellet in the Dissociation Solution.
12. Incubate the conical tube in the water bath at 37°C for 1 to 2 minutes to dissociate the organoids into fragments. NOTE: Incubation time will vary based on the size of organoids, however, do not incubate for longer than 2 minutes, as this may result in dissociation of organoids into single cells, leading to decreased seeding efficiency.
13. Dilute with at least 2 times Advanced DMEM/F12 medium to wash.
14. Centrifuge to pellet the dissociated organoids at 300 x g for 5 minutes at 4°C.
15. Aspirate the supernatant, and adjust seeding density by suspending the pellet in Complete Organoid Growth Medium.

Adjust Cell Density

The seeding density depends on the size and density of organoids cultured on the 24 well plate. Use of 2 to 3 wells of organoids per chip has been recommended.

To calculate volume for suspension of dissociated organoids:

Chip Seeding Volume = 35 µL

Number of chips = 6

Volume of media required to re-suspend dissociated organoids = (35 µL) x (6 chips) = 210 µL

Dilute the organoids with warm Complete Organoid Growth Medium to the required final cell density.

Seed Fragmented Organoids to Top Channel

Work with one chip at a time. After seeding the first chip, assess cell density within the channel through the microscope, adjusting the density of the cell suspension accordingly for the subsequent chips if necessary.

1. Bring the 150 mm dish containing the prepared and washed ECM-coated chips to the BSC.
2. Avoiding contact with the ports, carefully aspirate excess medium droplets from the surface of one chip.
3. Very gently, agitate the cell suspension before seeding each chip to ensure a homogeneous mixture for even seeding.
4. Quickly and steadily, pipette 35 µL of the cell suspension into the top channel inlet port while aspirating the outflow fluid from the chip surface near the outlet. Avoid direct contact of the aspirator tip with the outlet port.
5. Cover the dish and transfer to the microscope to check the seeding density within the chip. Note: At the optimal seeding density, the organoid fragments will form an even cell layer on the top channel of the chip (See Figure 4).
6. If seeding density is not optimal, return the chips to the BSC, and wash the channel with 200 µL of fresh medium 2 times. Do not aspirate the medium from the channel. Adjust cell density accordingly, and repeat steps 3 through 5 until the correct density is achieved within the channel.
7. After confirming the correct cell density, seed cells in the remaining chips. Note: Minimize the amount of time the cells are outside the incubator by seeding no more than 12 chips at a time, and immediately placing the chips in the incubator at 37°C after seeding each batch of 12 chips.
8. Place the dish of chips (with the DPBS reservoir) at 37°C and incubate undisturbed overnight.

Note: A minimum 6 hours is required for the organoid fragments to fully attach to the chip membrane surface.

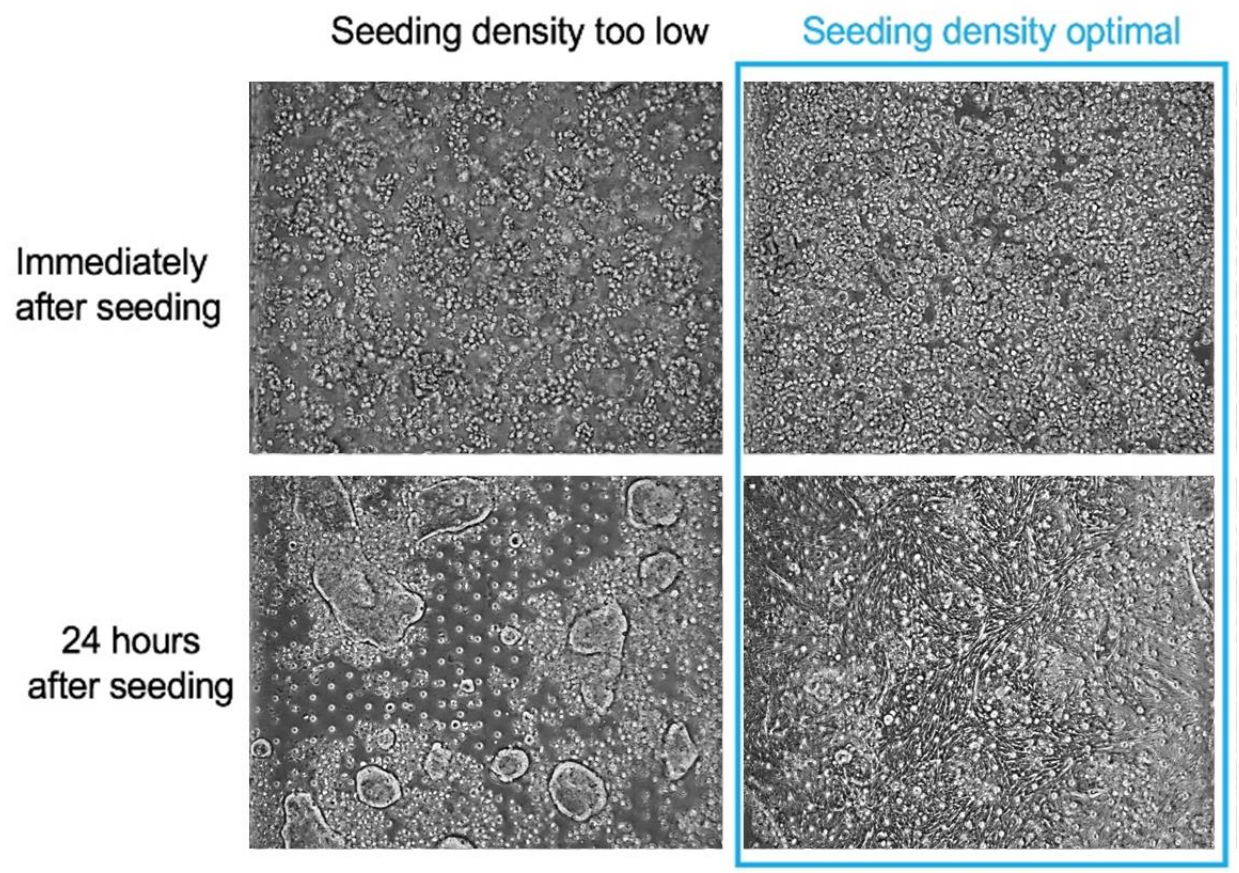


Figure 4: Organoid fragment seeding density examples

Day 1: Chips to Pods and Pods to Zoë

Goals:

- De-gas and equilibrate media
- Connect chips to Pods
- Connect Pods to Zoë

Key Steps:

- Gas equilibration of media
- Prime Pods
- Wash chips
- Connect chips to Pods
- Connect Pods to Zoë and run Regulate

Required Materials:

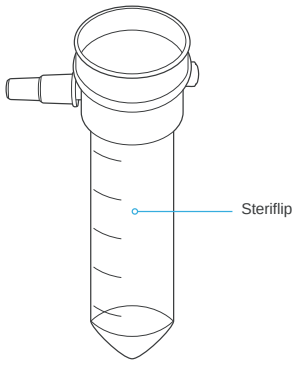
- Complete Organoid Growth Medium
- Installed and qualified Zoë
- Prepared chips
- Pods (sterile) — 1 per chip
- Tray — 1 per 6 chips
- Steriflip® filtration unit: PVDF filter 0.45 µm (sterile)
- Vacuum source (minimum -70 kPa)
- Serological pipettes
- Pipettors and filtered tips
- 37°C water or bead bath
- 70% ethanol

Gas Equilibration of Media

The media equilibration step is very important to the success of Organ-Chip culture. Omission of this step will cause the eventual formation of bubbles in the chip, the Pod, or both, which will in turn cause irregular flow and negatively impact cell viability. Minimize the time that medium is outside of a warmed environment (no more than 10 minutes), such as an incubator or bath, as gas equilibrium can become compromised when medium is allowed to cool.

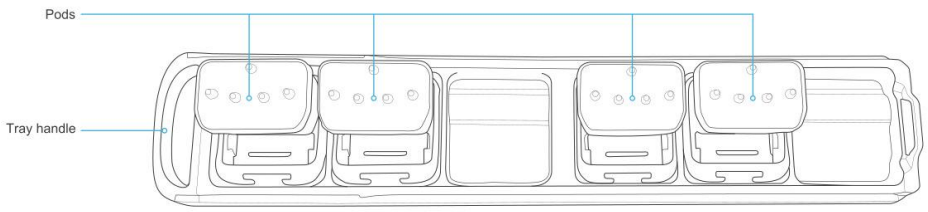
1. Prepare 50 mL of complete Complete Organoid Growth Medium per 6 chips in separate 50 mL conical tubes. Note: Approximately 7 mL of medium will be used per chip.
2. Warm the 50 mL conical tubes of media at 37°C in a water or bead bath for at least 1 hour.
3. Immediately connect the 50 mL tube containing each warmed medium to a Steriflip® unit.
    1. Attach each conical tube containing warmed medium to a Steriflip® unit.
    2. With the unit "right-side-up" (medium in the bottom conical tube), apply vacuum for 10 seconds.
    3. Invert the Steriflip® -connected tubes, and check that the medium begins to pass from the top conical tube to the lower tube. Note: The vacuum source must operate at -70 kPa at minimum. At this correct pressure, it should take about 2 seconds for every 10 mL of medium to flow through the filter. If it takes longer, stop and see the troubleshooting protocol — medium not equilibrated properly.
    4. Leave the filtered medium under vacuum for 5 minutes.
4. Remove the vacuum tubing from the Steriflip® units.
5. Separate the conical tubes containing media from the Steriflip® unit and immediately place the conical tubes containing media in the incubator with the caps loose.

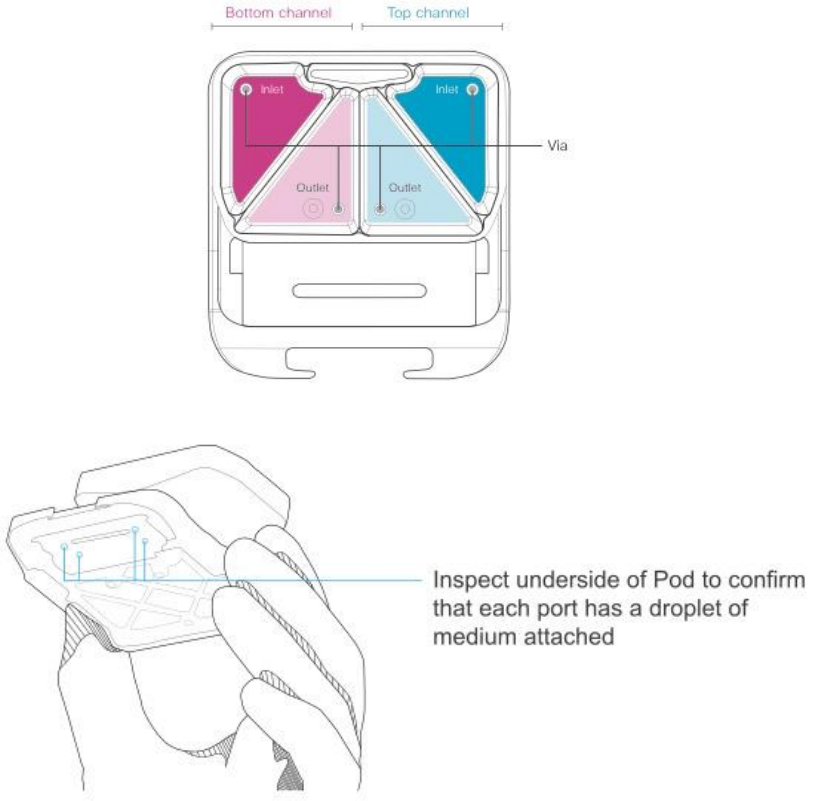
    As noted above, minimize the time that media is outside of the incubator during Pod preparation to maintain temperature. This is a very important step to ensure success of the chips.



Prime Pods

1. Sanitize the exterior of the Pod packaging and trays with 70% ethanol, wipe, and transfer to the BSC.
2. Open the Pod package and place the Pods into the trays. Orient the Pods with the reservoirs toward the back of the tray.
3. Place 3 mL of pre-equilibrated, warm Complete Organoid Growth Medium to the top and bottom inlet reservoirs of each Pod.
4. Pipette 300 µL of pre-equilibrated, warm Complete Organoid Growth Medium to the top and bottom outlet reservoirs of each pod, placing the media directly over each outlet via.
5. Bring trays containing Pods to the incubator and slide completely into Zoë with the tray handle facing outward.
6. Run the Prime cycle on Zoë.
    1. Use the rotary dial to highlight “Prime” on the display.
    2. Press the rotary dial to select “Prime.”
    3. Rotate the dial clockwise to highlight “Start.”
    4. Press the dial again to select “Start” and begin. Note: Once “Start” is selected, there will be an audible sound as Zoë engages the Pods.
7. Close the incubator door, and allow Zoë to prime the Pods; this process takes approximately 1 minute. Note: When the status bar reads “Ready,” the “Prime” cycle is complete.
8. Remove the tray from Zoë, and bring to the BSC.
9. Verify that the Pods were successfully primed. This is very important for success.
    1. Inspect the underside of each Pod — look for the presence of small droplets at all four fluidic ports. Droplets will vary in size from a small meniscus to larger droplets; often droplets on the outlet ports will be larger.
    2. If any Pod does not show droplets, re-run the “Prime” cycle on those Pods.
    3. If any media dripped onto the tray (this may occur more often by the outlet ports), clean tray with a wipe sprayed with 70% ethanol.
10. Once it is confirmed that all Pod ports are wet with droplets, put the tray of Pods to the side in the BSC.





Wash Chips

1. Transfer the seeded chips in a 150 mm dish from the incubator to the BSC.
2. Gently wash the top channel of each chip with warm, equilibrated Complete Organoid Growth Medium, aspirating the outflow from the chip surface.
3. Gently wash the bottom channel of each chip with warm, equilibrated Complete Organoid Growth Medium, aspirating outflow from the chip surface.
4. Place small droplets of equilibrated Complete Organoid Growth Medium on all inlet and outlet ports.
5. Repeat steps 2 through 4 for each chip.

Connect Chips to Pods

1. Holding one chip (while it remains in the chip carrier) in the dominant hand and one Pod firmly in the non-dominant hand, slide the chip carrier into the tracks on the underside of the Pod until the chip carrier has seated fully.
2. Place thumb on the chip carrier tab and gently, but firmly, depress the tab in and up to engage the tab of the chip carrier with the Pod.
3. Aspirate any excess medium on the chip surface from the Pod window.
4. Place the Pod onto the tray.
5. Repeat steps 1 through 4 for each Pod and chip carrier.
6. Confirm that there is sufficient media in each Pod inlet and outlet reservoirs and that the Pod lids are flat and secure.

Connect Pods to Zoë and Run Regulate

1. Place trays that are holding Pods and chips immediately into Zoë to prevent media from cooling and losing its gas equilibration.
2. Program the appropriate Organ-Chip culture conditions on Zoë. These conditions will start as soon as the Regulate cycle is complete. For the Duodenum Intestine-Chip, set the flow rate to 30 µL / hour for both top and bottom channels
3. Run Regulate cycle.
    1. Using the rotary dial, highlight the “Regulate” field.
    2. Press the dial to select “Regulate,” and rotate the dial clockwise to “Start.”
    3. Press the dial again to select “Start” and begin the Regulate cycle. Note: Once “Start” is selected, there will be an audible sound as Zoë engages the Pods.
    4. At this point the “Activation” button will glow blue.
    5. The Regulate cycle lasts 2 hours. After the cycle has finished, Zoë will begin flow at the preset Organ-Chip culture conditions. To cancel the Regulate cycle (only if needed), on Zoë, select the “Regulate” field with the dial and press the button to select. Rotate the dial counter-clockwise to select “Cancel.” Press the dial once more and wait 1 minute for the cycle to end, after which the tray can be removed. If cancelled, always rerun a complete Regulate cycle before proceeding.

Day 2: Maintain Chips in Zoë; Thaw Human Intestinal Microvascular Endothelial Cells (HIMECs)

Goals:

- Maintain chips in Zoë
- Cell culture inspection
- Expand HIMECs in flask prior to chip seeding as per established protocols

Key Steps:

- Via wash Pods
- Prepare HIMEC culture medium
- Prepare T75 flask
- Thaw and plate HIMECs

Required Materials:

- Complete HIMEC Culture Medium (at 37°C)
- 15 mL conical tube
- Attachment Factor
- T75 flask
- Serological pipettes
- Pipettes and tips
- Aspirator
- Centrifuge
- Chips in Pods
- 70% ethanol
- Microscope

Via Wash Pods

1. The day after connecting chips and Pods to Zoë, pause the Zoë by pressing the silver “Activation” button located above the tray bays. This stops flow and releases the Pods.
2. Slide the tray out of the bay and transfer to the BSC.
3. Remove the Pod lids. Using a 200 µL pipette, perform a via wash on each Pod inlet and outlet reservoir:
    1. Using the media within the Pod reservoir, pipette 200 µL of media at an angle over the via to dislodge any bubbles that may have formed at top of the via.
    2. Repeat this wash step for each of the four Pod reservoirs.
4. Replace Pod lids and return the trays to Zoë.

Prepare HIMEC Culture Medium

Reagent	Volume	Conc. [Stock]	Conc. [Final]	Source	Cat. No.
Endothelial Cell Basal Medium	500 mL	-	-	PromoCell	C-22121
Endothelial Cell Growth Medium MV2 Supplement Pack	-	-	-	PromoCell	C-39221
Primocin™	500 uL	50 mg / mL	50 µg / mL	InvivoGen	ANT-PM-1

- Endothelial Cell Growth Medium (C-22121) is supplied as a kit containing Endothelial Cell Basal Medium (C-22221) and Endothelial Cell Growth Medium MV2 Supplement Pack (C-39221).
- Aseptically transfer all components of Endothelial Cell Growth Medium MV2 Supplement Pack to the Endothelial Cell Basal Medium bottle.
- Primocin is a non-toxic antimicrobial agent for primary cells.
- Store complete media at 4°C for up to 30 days.

Prepare T75 Flask

1. Warm a sufficient amount of Complete HIMEC Culture Medium to 37°C.
2. Label the culture flask with relevant information (e.g., cell type, passage number, date, initials).
3. Pipette Attachment Factor onto the growth surface of flask ensuring full coverage. (5 mL of Attachment Factor is used per T75 flask.)
4. Ensure that the coating solution covers the culture surface of the flask evenly. Incubate at room temperature for 5 minutes.
5. Aspirate excess coating solution after incubation.
6. Add 15 mL of Complete HIMEC Culture Medium to the flask, and store in the 37°C incubator until ready for plating.

Thaw and Plate HIMECs

HIMECs require prior one passage of expansion in culture after thawing before seeding in the chip.

1. Thaw the vial(s) of cells by immersing in a 37°C water bath for approximately 60 to 90 seconds. Closely observe the thawing process while gently agitating and remove the vial from the water bath just before the last ice pellet disappears.
2. Spray vial(s) with 70% ethanol and wipe dry prior to placing them in the BSC.
3. Immediately transfer the contents of the vial using a P1000 pipette into the prepared flask containing warm Complete HIMEC Culture Medium.
4. Rinse the vial with 1 mL of Complete HIMEC Culture Medium and add to the flask.
5. Incubate the flask in the incubator at 37°C for 6 hours.
6. Aspirate medium and carefully add 15 ml of fresh Complete HIMEC Culture Medium.
7. Return the flask back to the incubator at 37°C and 5% CO_2 overnight.
8. Alternatively, add 30 mL of complete HIMEC culture media to the flask. Leave the flask in the incubator at 37°C and 5% CO_2 overnight, followed by a media replenishment the next day.
9. Exchange medium in flask with fresh Complete HIMEC Culture Medium the following day, and every other day until use for seeding in the chip.

Day 3: Exchange Media in Chips and HIMEC Cultures

Goals:

- Maintain chips in Zoë
- Inspect chips
- Inspect HIMEC cultures
- Change medium in chips and Pods to Organoid Maintenance Medium (without Y-27632 or CHIR99021)
- Exchange medium in HIMEC flask

Key Steps:

- Prepare organoid maintenance medium
- Sampling and media replenishment
- Maintain HIMECs in culture

Required Materials:

- Chips in Pods
- Organoid Maintenance Medium
- Complete HIMEC Culture Medium
- Microscope

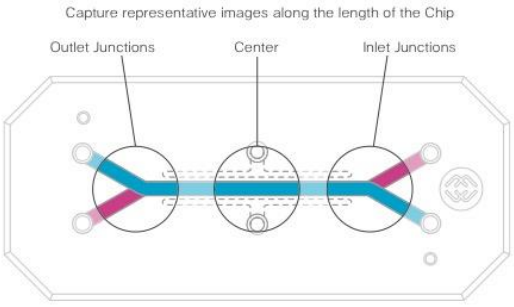
Prepare Organoid Maintenance Medium

Reagent	Volume	Conc. [Stock]	Conc. [Final]	Source	Cat. No.
IntestiCult™ OGMH Component A	50 mL	-	-	StemCell Technologies	06010 (kit); 06011 (part)
IntestiCult™ OGMH Component B	50 mL	-	-	StemCell Technologies	06010 (kit): 06012 (part)
Primocin	200 µL	50 mg / mL	100 µg / mL	Invivogen	Ant-pm-1

- Once organoid fragments are seeded and established in the chips, the cells can be cultured in Organoid Maintenance Medium, prepared without Y-27632 or CHIR99021 supplementation.
- IntestiCult™ Organoid Growth Medium (Human) (06010) is supplied as a kit containing IntestiCult™ OGM Human Component A (06011) and IntestiCult™ OGM Human Component B (06012).
- Primocin is a non-toxic antimicrobial agent for primary cells.
- Store prepared Organoid Maintenance Medium at 4°C for up to 7 days.

Sampling and Media Replenishment

1. Pause Zoë by pressing the silver “Activation” button.
2. Remove the trays and place in the BSC.
3. Inspect each chip for bubbles by eye.
4. Using a microscope, inspect cells in the chips to assess morphology and viability. Capture representative images at 10X or 20X magnification at the following locations:
   - Inlet junction
   - Center of channel
   - Outlet junction



5. In the BSC, remove the Pod lids and collect effluent medium from Pod outlet reservoirs for analysis if desired; avoid disturbing the Pod reservoir vias.

6. Gently aspirate any remaining medium from all Pod inlets and outlets, ensuring that a thin liquid film still covers the reservoir vias to avoid trapping air at the via surface.
7. Refill the Pod inlet reservoirs with fresh Organoid Maintenance Medium (without Y-27632 or CHIR99021) and perform a via wash:
    - Using medium within the Pod reservoir, pipette 1 mL of medium directly over the top of the via to dislodge any bubbles that may be present.
8. Replace the Pod lids and return trays to Zoë.
9. Press the silver “Activation” button to resume pre-set Organ-Chip culture conditions.
    - Zoë will engage when the “Activation” button glows blue.

Maintain HIMECs in Culture

1. Using a microscope, inspect HIMECs in the flask to assess morphology and confluency.
2. Aspirate medium from the flask and replace with 15 mL of fresh Complete HIMEC Culture Medium.
3. Return the flask to incubator and culture at 37°C and 5% CO_2 overnight.

Day 6: Seed HIMECs to Chip; Chips to Pods and Pods to Zoë; Add 2% Stretch

Goals:

- Harvest HIMECs and seed in chip
- De-gas and equilibrate media
- Connect chips to Pods
- Connect Pods to Zoë
- Set stretch to 2%, 0.20 Hz
- Culture under flow

Key Steps:

- Prepare counting solution
- Prepare chips
- Harvest HIMECs
- Seed HIMECs to bottom channel
- Gravity wash chips
- Gas equilibration of media
- Prime Pods
- Chips to Pods
- Pods to Zoë
- Add 2% stretch

Required Materials:

- Complete HIMEC Culture Medium (at 37°C)
- Organoid Maintenance Medium
- DPBS (at room temperature)
- Aspirator and sterile tips
- 15 mL conical tubes
- Trypan blue counting solution
- Hemocytometer
- 150 mm culture dishes (1 dish per 6 chips)
- Autoclaved chip cradle (1 cradle per 6 chips)
- Organoid chips (already in culture)
- Steriflip® filtration units: PVDF filter 0.45 µm (sterile)
- Vacuum source (minimum -70 kPa)
- Serological pipettes
- Pipettors and filtered tips
- 37°C water or bead bath
- 70% ethanol

Prepare Counting Solution

Trypan Blue Cell Counting Solution

Reagent	Volume	Source	Cat. No.
HIMEC Culture Medium	400 µL	Refer to page 35 for recipe	-
Trypan blue	50 µL	Sigma	93595

- Maintain counting solution at room temperature.
- Prepare in Eppendorf tube fresh for each use.

Prepare Chips

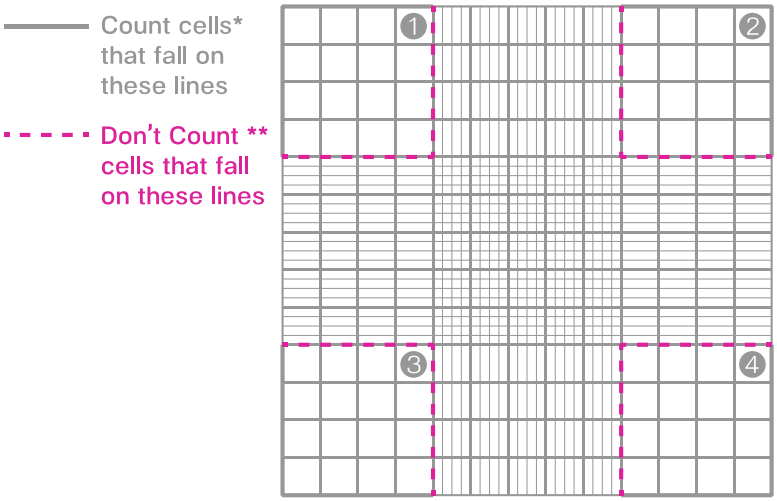
1. Pause the Zoë by pressing the silver “Activation” button located above the tray bays. This stops flow and releases the Pods.
2. Slide the tray out of the bay and transfer to the BSC.
5. Detach the chip and carrier from the Pod. To do this, hold the Pod in the non-dominant hand, place thumb on the chip carrier tab and gently, but firmly, depress the tab in and pull down to disconnect the tab of the chip carrier with the Pod. Slide the chip carrier out of the tracks on the underside of the Pod until the chip carrier has been disconnected fully.
6. Place the chips back into a sterile 150 mm dish.
7. Aspirate the media from the Pods, and place the Pods in trays back in the Zoë (without flow) until ready for reconnection.
5. Gently wash the bottom channel of the chip with 200 µL of Complete HIMEC Culture Medium. Aspirate the medium outflow at the surface of the chips, leaving the medium in the channels.
6. Cover the 150 mm dish and place the chips in the incubator until the cells are ready for seeding.

Harvest HIMECs

HIMECs that have been expanded in culture must be harvested and counted for bottom channel seeding. Typically HIMECs are adjusted to a density range of 8 x 10^6 cells / mL to achieve a complete monolayer prior to seeding in the bottom channel.

1. Bring the culture flasks containing HIMECs from the incubator into the BSC.
2. Aspirate culture media and add 15 mL of DPBS to wash the culture surface. Aspirate the DPBS wash.
3. Add 3 mL of TrypLE to the flasks. Incubate for 2 to 3 minutes at 37°C.
4. Tap the side of the flask gently, and inspect the culture under the microscope to assess complete detachment of cells from the culture surface.
5. Add 9 mL of warm Complete HIMEC Culture Medium to the flasks and pipette gently to mix, while collecting all cells from the culture surface of the flasks.

6. Transfer the contents of the flasks into a sterile 15 mL conical tube.
7. Add complete HIMEC culture medium to bring the total volume of the tube to 15 mL.
8. Centrifuge the cells at 300 x g for 5 minutes.
9. Carefully aspirate the supernatant, leaving approximately 100 µL of medium above the cell pellet. Note: The cell pellet will be very small. Aspirate carefully.
10. Loosen the cell pellet by flicking the tube gently.
11. Using a P1000 pipette, gently resuspend the cells by adding 200 µL of complete HIMEC culture medium.
12. Pipette gently to create a homogeneous mixture, and transfer 50 µL of the cell suspension to the trypan blue cell counting solution (This will yield a dilution factor of 10).
13. Mix the counting solution thoroughly and count cells using a manual hemocytometer.



Cell Counting and Viability Assessment

a. Count both viable and non-viable cells in each quadrant of the hemocytometer.
Live Cell Count; Dead Cell Count; Total Cell Count

b. Calculate percent viability of the cell solution.
(Live Cells) ÷ (Total Cells) x 100 = % Viability

c. Calculate viable cell concentration. The dilution factor is 10 when prepared in the trypan blue solution above.
(Live Cell Count x 10 x 10^4) ÷ 4 = Viable Cell Concentration (cells / mL)

d. Calculate viable cell yield.
(Viable Cell Concentration) X (Cell Suspension Volume) = Viable Cell Yield (cells)

14. Dilute the HIMECs to 8-10 x 10^6 cells / mL in Complete HIMEC Culture Medium.

Seed HIMECs to Bottom Channel

Work with one chip at a time. After seeding the first chip, invert the chip and assess cell density within the channel through the microscope, adjusting the density of the cell suspension accordingly for the next chips if necessary.

1. Bring the 150 mm dish containing the prepared chips to the BSC.
2. Avoiding contact with the ports, carefully aspirate excess medium droplets from the surface of one chip.
3. Gently agitate cell suspension before seeding each chip to ensure a homogeneous cell suspension.
4. Seed 15 to 20 µL of the HIMEC suspension into the bottom channel of one chip first, while aspirating the outflow.
5. Cover the dish and transfer to the microscope to check the seeding density within the chip.
6. If seeding density is not optimal, return the chip to the BSC and wash the channel with 200 µL of fresh medium 2 times. Do not aspirate the medium from the channel. Adjust cell density accordingly, and repeat steps 3 through 5 until the correct density is achieved within the channel. Note: Correct seeding density is essential for success of the chip cultures.
7. After confirming the correct cell density, seed cells in the remaining chips, invert each chip, and rest the edge of the chip carrier on the chip cradle. Note: Each chip cradle can support up to 6 chips inside a 150 mm cell culture dish. (See Figure 6.)
8. Place the small reservoir (15 mL conical tube cap containing sterile DPBS) inside the 150 mm dish to provide humidity for the cells. Replace dish lid. Note: Minimize the amount of time the cells are outside the incubator by seeding no more than 12 chips at a time, and immediately placing the chips in the incubator at 37°C after seeding each batch of 12.
9. Place the chips still in the dish in the 37°C incubator for approximately 2 hours, or until cells in the bottom channel have attached.
10. Once HIMECs have attached (approximately 2 hours post-seeding), flip the chips back to an upright position. Note: Remove the chip cradle from the dish, wipe with 70% ethanol to clean, and autoclave for use in next experiment.

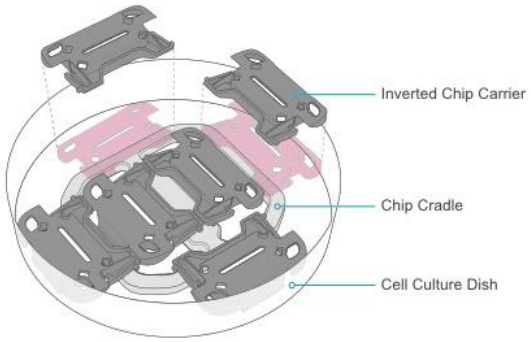


Figure 6: Inverting chips during endothelial attachment

Gravity Wash Chips

A gentle gravity wash is performed 2 hours post-seeding, after the HIMECs in the chip have attached, to ensure that nutrients are replenished and the channels do not dry out. During a gravity wash the medium should be observed to flow through the channel and outflow from the outlets.

1. With a P200 pipette, gently wash the top channel of each chip with warm, equilibrated complete Organoid Maintenance Medium to remove any possible bubbles in the channel.
2. Repeat the step using complete HIMEC Culture medium to wash the bottom channel.
3. If the media does not flow through the channel, very gently pipette a small amount of medium into the inlets, until a small droplet appears on the outlet, or until a bubble is ejected through the outlet.
4. Place additional droplets of media to fully cover all inlet and outlet ports. (See Figure 7.)

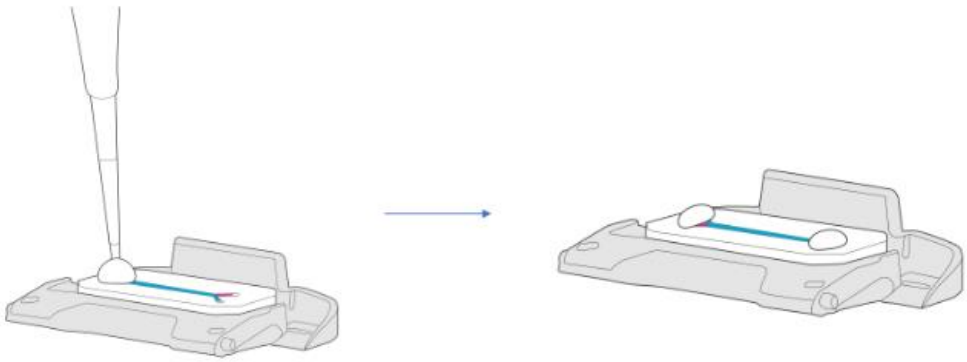


Figure 7. Chip with medium drops covering the inlet and outlet ports

Gas Equilibration of Media

1. Prepare 50 mL of Organoid Maintenance Medium per 12 chips in a 50 mL conical tube. Note: At least 3.3 mL of Organoid Maintenance Medium will be used per chip.
2. Prepare 50 mL of Complete HIMEC Culture Medium per 12 chips in a 50 mL conical tube. Note: At least 3.3 mL of Complete HIMEC Culture Medium will be used per chip.

Refer to page 30: Gas Equilibration of Media and follow same process as listed.

Prime Pods

1. Place the Pods in trays from the Zoë (without flow) into the BSC.
2. Pipette 3 mL of pre-equilibrated, warm Organoid Maintenance Medium to the top channel inlet reservoir of each Pod.

3. Pipette 300 µL of pre-equilibrated, warm Organoid Maintenance Medium to each outlet reservoir, placing the media directly over each outlet via.
4. Pipette 3 mL of pre-equilibrated, warm Complete HIMEC Culture Medium to the bottom channel inlet reservoir of each Pod.
5. Pipette 300 µL of pre-equilibrated, warm Complete HIMEC Culture Medium to each outlet reservoir, placing the media directly over each outlet via.
6. Bring trays containing Pods to the incubator and slide completely into Zoë with the tray handle facing outward.
7. Run the Prime cycle on Zoë.

Refer to page 31: Prime Pods for more information.

Chips to Pods

Refer to page 30: Chips to Pods and follow same process as listed.

Pods to Zoë

Refer to page 33: Pods to Zoë and follow same process as listed.

Add 2% stretch

1. Using the rotary dial, highlight the “Stretch” field.
2. Press the dial to select “Stretch” and rotate the dial clockwise to increase stretch to “2%”.
3. Press the dial to select “Freq.” and rotate the dial clockwise to increase stretch to “0.2 Hz”.
4. Press the “Activation” button.

Day 7: Maintenance of Chips in Zoë; Increase Stretch to 10%

Goals:

- Maintain chips in Zoë
- Inspect cells in chips
- Set stretch to 10%, 0.20 Hz

Key Steps:

- Via wash Pods
- Increase stretch to 10%, 0.2 Hz

Required Materials:

- Pipettes and tips
- Chips in Pods
- Microscope

Via Wash Pods

1. The day after connecting chips and Pods to Zoë, which begins the process of Organ-Chip culture, pause the Zoë by pressing the silver “Activation” button located above the tray bays. This stops flow and releases the Pods.
2. Slide the tray out of the bay and transfer to the BSC.
3. Remove the Pod lids. Using a 200 µL pipette, perform a via wash on each Pod inlet and outlet reservoir.
4. Using media within the Pod reservoir, pipette 200 µL of media directly over the top of the via to dislodge any bubbles that may be present.
5. Repeat this wash step for each of the four Pod reservoirs.
6. Replace Pod lids and return the trays to Zoë.

Increase Stretch to 10%, 0.2Hz

1. Using the rotary dial, highlight the “Stretch” field.
2. Press the dial to select “Stretch” and rotate the dial clockwise to increase stretch to “10.0%”.
3. Press the dial to select “Freq.” and rotate the dial clockwise to increase stretch to 10% and the frequency to 0.2 Hz.
4. Press the “Activation” button.

Day 8+: Chip Maintenance and Sampling

Goals:

- Maintain chips in Zoë
- Cell culture inspection
- Collect samples for analysis

Key Steps:

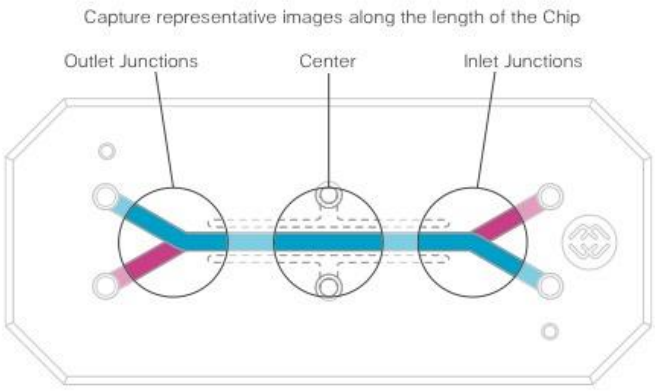
- Sampling and media replenishment

Required Materials:

- Chips in Pods
- Cell culture media

Sampling and Media Replenishment

1. Pause Zoë by pressing the silver “Activation” button.
2. Remove the trays and place in the BSC.
3. Inspect each chip for bubbles by eye.
4. Using a microscope, inspect cells in the chips to assess morphology and viability. Capture representative images at 10X or 20X magnification at the following locations:
   - Inlet junction
   - Center of channel
   - Outlet junction



5. Remove the Pod lids and collect effluent medium from Pod outlet reservoirs for analysis.
   - Collect effluent from the indicated regions; avoid disturbing the Pod reservoir vias.
6. Gently aspirate any medium not collected for analysis, ensuring that a thin liquid film still covers the reservoir vias so that no air is introduced into the vias.
7. Refill the Pod media reservoirs with appropriate fresh cell culture medium and perform a via wash by pipetting 1 mL of medium directly over the top of the via to dislodge any bubbles that may be present.
8. Replace the Pod lids and return trays to Zoë.
9. Press the silver “Activation” button to resume pre-set Organ-Chip culture conditions. Zoë will engage when the “Activation” button glows blue.

Troubleshooting

Issue	Section	Step	Recommendation
Bubbles are present in channel	Chip Preparation	Introduce ER-1 Solution to Channels; Activate Chips; Coat Chips with ECM	Dislodge by washing the channel with appropriate solution until all bubbles have been removed. If bubbles persist, it may be helpful to aspirate the channel dry and slowly re-introduce the solution.
Bubbles in the ports upon introduction of media into the chip	Chips to Pods and Pods to Zoë	Wash Chips	Since the chip material is hydrophobic, bubbles could get trapped at the ports. Dislodge bubbles using pipette tip or aspirate the channels and reintroduce equilibrated media.
Media take too long to pass through Steriflip®	Chips to Pods and Pods to Zoë	Equilibration of Media	Vacuum pressure is not reaching -70kPa. Need to find an alternate vacuum source.
Pods do not prime	Chips to Pods and Pods to Zoë	Prime Pods	Ensure that medium is directly over all Pod vias. If problem persists, record the Pod lot number and replace with new a Pod.
Screen is frozen or unresponsive	Chips to Pods and Pods to Zoë; Maintaining and Sampling	Pod to Zoë; Maintenance and Regulate	Power off Zoë and then turn Zoë on again; contact our support team if problem persists.
Pods stuck in Zoë	Maintaining and Sampling	Maintenance and Regulate	Pod lid not secured on Pod. Try wiggling tray to the right and left as you slide it out while keeping it level. If problem persists, contact our support team.
Pods not flowing properly or evenly; Bubbles observed in chip	Maintaining and Sampling	Maintenance and Regulate	There is inherent variability with Zoë, however large fluctuations and major flow issues primarily result from bubbles. Remove chip from Pod; flush chip with media; re-prime Pod with degassed media; connect chip to Pod; and run the Regulate cycle.

Potential Root Causes of Bubbles

Check the following if a high failure rate due to bubbles is observed or if bubbles are persistent, despite following the mitigation steps above.

- Medium not sufficiently equilibrated before adding to Pods: be sure to follow media preparations steps in section Gas Equilibration of Media.
- Vacuum for Steriflip® too weak: ensure that media is passing through the Steriflip® in about 10 seconds.
- Incorrect Steriflip® used: confirm correct Steriflip® unit (Millipore SE1M003M00).
- Medium not warmed correctly before Steriflip® step: Be sure to follow media preparations steps in section Gas Equilibration of Media.
- Insufficient priming: disconnect chip and reprime Pod.

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