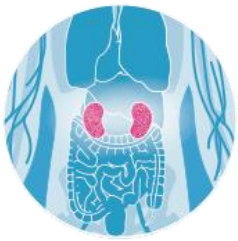
emulate



Proximal Tubule Kidney-Chip Co-Culture Protocol

July 16, 2019

EP169 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™ Stretchable Chip, 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 the tissue. The Orb delivers a mix of 5% CO_2, vacuum stretch, and power for up to four Zoës.

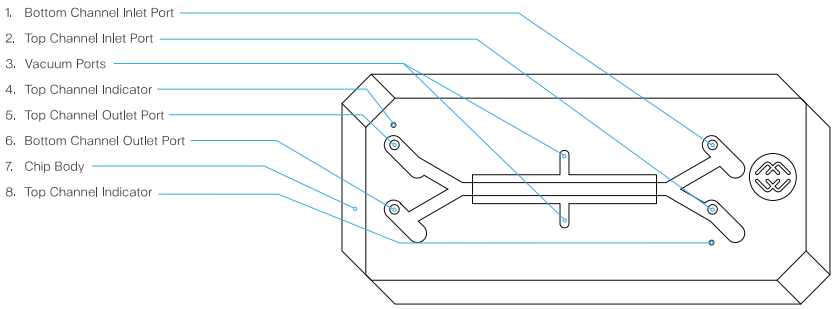
Organ-Chip (Chip-S1™)

Our Chip-S1™ can be configured to emulate 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 Proximal Tubule Kidney-Chip.

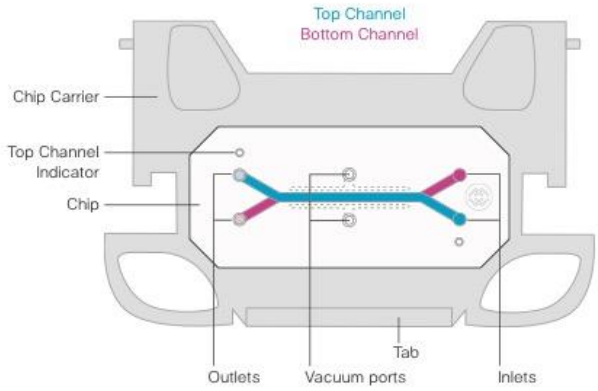
The microenvironment created within each Chip-S1 includes epithelial cells in the top channel and endothelial cells in the bottom channel. Top and bottom channels are separated by a porous membrane that allows for cell-cell interaction like those that are seen *in vivo*. These two channels are fluidically 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.

Organ-Chip S-1™

Configuration



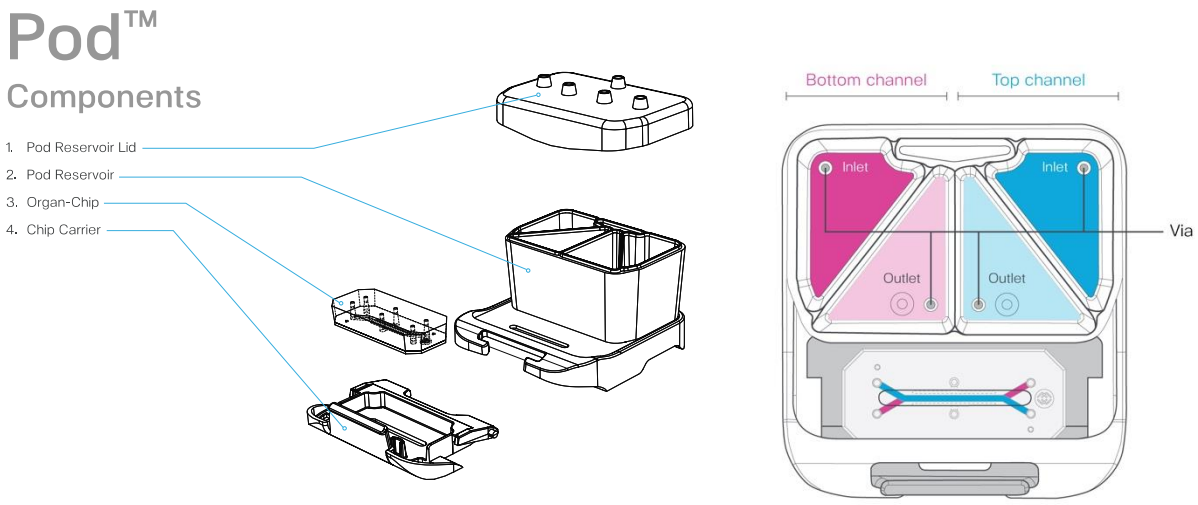
Chips are supplied pre-packaged in the chip carrier and should be maintained in the chip carrier at all times. The chip carrier is designed to hold chips in place, eliminating the need to disturb or distort the chip during preparation. In addition, the chip carrier connects securely to the Pod, which 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.)



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.

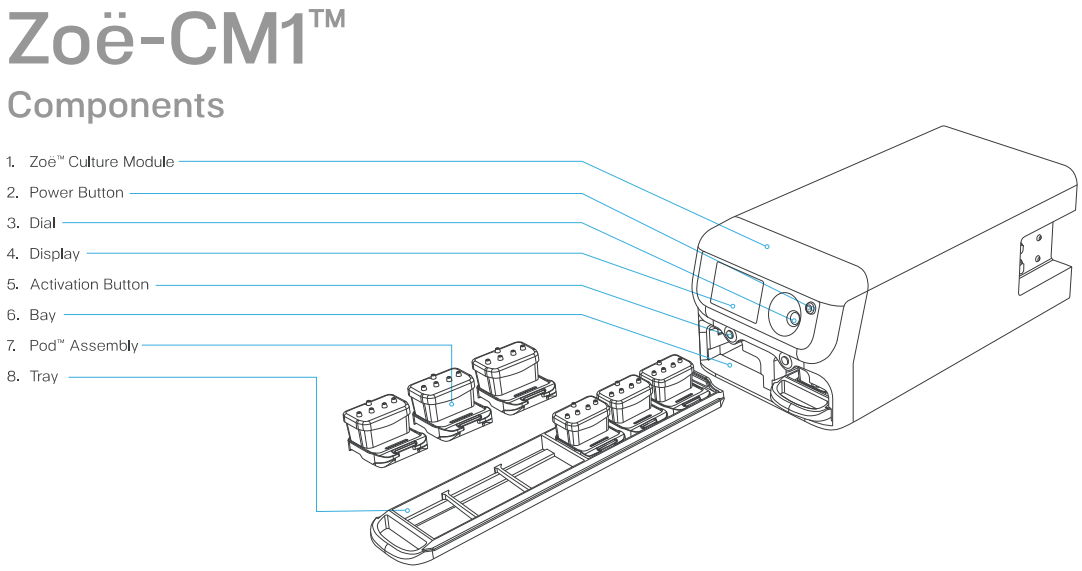
The Pod's reservoirs allow users to introduce nutrient media or blood, precisely control dosing to test drugs or other inputs, and sample chip effluent. Each Pod reservoir holds up to 4 mL.



Zoë™ Culture Module

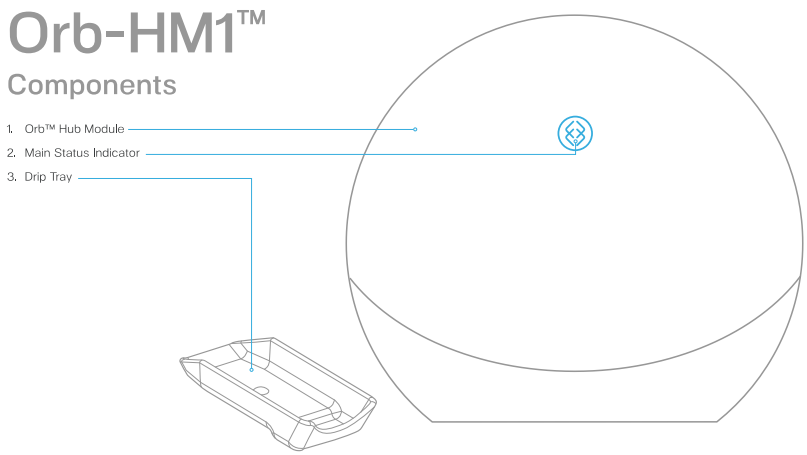
The Zoë™ Culture Module is designed to sustain the life of cells within Emulate's Organ-Chips. It provides the dynamic flow of media and the mechanical forces that help recreate the microenvironment cells experience *in vivo*. The instrument automates the precise conditions needed for simultaneous cell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

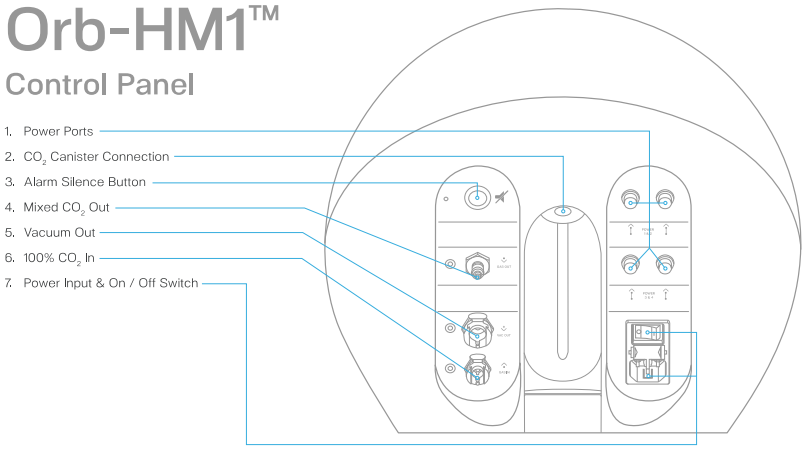
Zoë enables independent control of the flow rate of media through the top and bottom channels of the Organ-Chips, as well as stretch parameters — including frequency and amplitude — of the Organ-Chip. Additionally, Zoë has automated algorithms to prime the fluidic channels of Pods with media, and programming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



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 Proximal Tubule Kidney Bio-Kit includes pre-qualified primary human kidney cells. Cells are shipped in a cryogenic storage vacuum flask and must be stored in liquid nitrogen until use.

The Proximal Tubule Kidney-Culture Bio-Kit includes the following cells:

Renal tubular epithelial cells (top channel):
Human Renal Proximal Tubule Epithelial Cells (hRPTECs)

Vascular endothelial cells (bottom channel):
Human Renal Microvascular Endothelial Cells (hRMVECs)

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 (ECM, Matrigel®)

Day -3: Thaw Cells

- Part I: Thaw human Renal Microvascular Endothelial Cells (hRMVECs)
  - Prepare hRMVECs Culture Media and flask
  - Thaw and plate hRMVECs
- Part II: Thaw human Renal Proximal Tubule Epithelial Cells (hRPTECs)
  - Prepare hRPTECs Culture Media and flask
  - Thaw and plate hRPTECs

Day -1: Chip Preparation

- Prepare chips
- Prepare ER-1™ solution
- Introduce ER-1™ solution to channels
- Activate chips
- Prepare ECM solution
- Coat chips with ECM

Day 0: hRMVECs and hRPTECs to Chip

- Prepare maintenance medium for both cell types and warm at 37˚C
- Prepare chips
- Prepare hRMVECs for seeding
- Seed hRMVECs to the bottom channel
- Flip chip upside-down using chip cradle
- Allow cells to attach (2.5 hrs post-seeding)
- Gravity wash bottom channel of chips
- Seed hRPTECs to top channel
- Allow cells to attach (2.5 hrs post-seeding)
- Gravity wash both channels and prepare chips for overnight static condition with pipette tips

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+: Maintaining and Sampling

- Maintenance and the Regulate cycle
- 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
Human Proximal Tubule Kidney Bio-Kit	Co-Culture 12- or 24-pack	Emulate	-
Zoë-CM-1™ Culture Module	1 per 12 chips	Emulate	-
Orb-HM1™ Hub Module	1 per 4 Zoë™	Emulate	-
Chip-S1™ Stretchable Chip	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
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	-	-
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	-	-
T25 flasks	-	-	-
T75 flasks	-	-	-
Ice bucket	-	-	-
70% ethanol and wipes	For surface sterilization	-	-

Reagents, Media & Supplements

Reagent	Description	Supplier	Catalog Number
ER-1™ reagent	5 mg powder	Emulate	ER105
ER-2™ buffer	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
Trypsin-EDTA Solution	0.05% Trypsin	Sigma	T3924
REGM™ Renal Epithelial Cell Growth Medium BulletKit™	Epithelial Growth Medium & Supplements	Lonza	CC-3190
REBM™ Basal Medium	Base Epithelial Grown Medium	Lonza	CC-3191
REGM™ SingleQuots™ Kit	Supplements and Growth Factors	Lonza	CC-4127
CSC Medium (Kit)	Endothelial Medium & Supplements	Cell Systems	4Z3-500
Culture Boost™	50X Supplement	Cell Systems	(4CB-500)
Attachment Factor™	1X	Cell Systems	(4Z0-210)
Cell Freezing Medium	1X	Cell Systems	4Z0-705
Matrigel®	LDEV-free	Corning	354234
Collagen Type IV	5 mg powder	Sigma	C5533
Penicillin-Streptomycin	10,000 U/mL; 10 mg/mL	Sigma	P4333
Fetal Bovine Serum (FBS)	Sterile Heat-inactivated	Sigma	F4135 or F8317
Human Serum	USA origin, sterile-filtered	Sigma	H4522

Fungin™ (optional)	10 mg / mL	InvivoGen	ANT-FN-1

Notes for ER-1™ reagent and ER-2™ buffer

- 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.

Notes for Fungin™

- Addition of 0.1% Fungin™ to any Proximal Tubule Kidney-Chip medium is optional to prevent fungal contamination.
- This antimycotic compound kills yeasts, molds, and fungi by disrupting ionic exchange through the cell membrane. This reagent has no effect on eukaryotic cell viability of function due to its mechanism of action.

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 during an experiment.

Cell Storage

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

Proximal Tubule Kidney-Chip Culture Protocol

Day X: Reagent Preparation

Aliquot Reagents

Aliquot reagents prior to use to avoid multiple freeze-thaw cycles of the stock solutions.

Collagen-IV (ECM)

Reagent	Conc. [Stock]	Amount	Volume	Solvent
Collagen-IV	1 mg / mL	5 mg	5 mL	DPBS

- Resuspend 5 mg collagen-IV in 5 mL of DPBS according to manufacturer's instructions.
- Aliquot to single-use volumes and store at -20°C.

Matrigel® (ECM)

Matrigel® bottle 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. (Do not only use ice — add some water to make it slushy, as this solution gels rapidly at temperatures above 10°C.) Use pre-chilled pipettes, tips, and tubes at -20°C prior to aliquoting.

- After thawing, aliquot Matrigel® to the desired volume (e.g.,100~200 µL) based on the specific stock concentration and the required volume per single experiment for the ECM coating step.
- Store aliquots at -20°C.

Day -3: Thaw hRMVECs and hRPTECs

Goals:

- Thaw and expand hRMVECs and hRPTECs in flasks prior to seeding in chips

Key Steps:

- Prepare hRMVEC Culture Medium and coated flask
- Prepare hRPTEC Culture Medium
- Thaw and Plate hRMVECs
- Thaw and Plate hRPTECs

Required Materials:

- Complete hRMVEC culture medium (at 37°C)
- Complete hRPTEC culture medium (at 37°C)
- 15 mL conical tube
- Attachment Factor™
- T-75 flask
- Serological pipettes
- Pipettes and tips
- Aspirator
- Centrifuge
- 70% ethanol

Part I. Prepare hRMVEC Culture Media and Flask

hRMVEC Culture Media

Base hRMVEC Culture Medium (500 mL)

Reagent	Volume	Conc. [Stock]	Conc. [Final]	Source	Cat. No.
CSC basal medium	485 mL	-	-	Cell Systems	4Z3-500
Culture-boost	10 mL	-	2%	Cell Systems	4CB-500
Pen / strep	5 mL		1%	Sigma	P4333

- Store at 4°C.
- Use within 30 days of preparation.

Complete hRMVEC Culture Medium (50 mL)

Reagent	Volume	Conc. [Stock]	Conc. [Final]	Source	Cat. No.
Base hRMVEC Culture Medium	45 mL	-	-	Recipe Above	-
FBS	5 mL	-	10%	Sigma	F4135

- Store at 4°C.
- Use within 7 days of preparation.

Prepare Flask

1. Warm sufficient amount of Complete hRMVEC Culture Medium and Attachment Factor™ to 37°C.
2. Label the culture flask with relevant information (e.g., cell type, passage number, date, initials).
3. Pipette Attachment Factor onto growth surface of flask ensuring full coverage. (5 mL of Attachment Factor is used per T75 flask.)
4. Place prepared flask into 37°C incubator to coat surface and maintain temperature until plating cells.

Thaw and Plate hRMVECs

Thawing and Maintaining Cells

1. Thaw the vial(s) of cells by immersing in a 37°C water bath, without submerging the cap. Closely observe while gently agitating and remove from the water bath just before the last of the ice disappears.
2. Once only a small ice pellet remains, immediately remove the vial from the water bath, wipe dry, spray vial(s) with 70% ethanol and wipe dry again prior to placing in the BSC.
3. Immediately transfer the contents of the vial into 3 mL of warm Complete hRMVEC Culture Medium in a sterile 15 mL conical tube.
4. Rinse the cryovial with 1 mL of Complete hRMVEC Culture Medium and collect in the 15 mL tube.
5. Bring the volume to 15 mL with Complete hRMVEC Culture Medium.
6. Centrifuge 200 x g for 5 minutes at room temperature.
7. Aspirate and discard supernatant, leaving approximately 100 µL of medium covering the pellet.
8. Loosen the cell pellet by gently flicking the tube.
9. Re-suspend cells in 15 mL of Complete hRMVEC Culture Medium.
10. Aspirate and discard excess Attachment Factor from the T75 flask that was prepared earlier. Note: Rinsing and / or drying the flask prior to adding cells is not necessary.
11. Add the hRMVECs suspension to the freshly coated T75 flask.
12. Incubate overnight at 37°C and 5% CO_2.
13. Exchange with fresh Complete hRMVEC Culture Medium every other day until use for seeding in the chip.

Part II. Prepare hRPTEC Culture Media and Flask

hRPTEC Culture Media

Base hRPTEC Culture Medium (500 mL)

Reagent	Volume	Conc. [Stock]	Conc. [Final]	Source	Cat. No.
REBM™ Renal Epithelial Cell Growth Basal Medium	492 mL	-	-	Lonza	CC-3191
REGM™ SingleQuots™ Kit containing:				Lonza	EpCC-4127
• Human Epidermal Growth Factor (hEGF)	0.5 mL	-	-	-	-
• Insulin	0.5 mL	-	-	-	-
• Hydrocortisone	0.5 mL	-	-	-	-
• Transferrin	0.5 mL	-	-	-	-
• Triiodothyronine	0.5 mL	-	-	-	-
• Epinephrine	0.5 mL	-	-	-	-
Pen/Strep	5 mL	-	1%	Sigma	P4333

- Note: Do not use gentamicin sulfate from REGM™ SingleQuots™ Supplement Pack.
- Store at 4°C.
- Use within 30 days of preparation.

Complete hRPTEC Culture Medium (50 mL)

Reagent	Volume	Conc. [Stock]	Conc. [Final]	Source	Cat. No.
Base hRPTEC Culture Medium	49.75 mL	-	-	Recipe Above	-
FBS	0.25 mL	-	0.5%	Lonza (from kit above)	EpCC - 4127

- Store at 4°C.
- Use within 7 days of preparation.

Prepare Flask

1. Label the culture flask with relevant information (e.g., cell type, passage number, date, etc.).

Thaw and Plate hRPTECs

Thawing and Maintaining Cells

1. Thaw the frozen vial(s) of cells by immersing in a 37°C water bath, without submerging the cap. Closely observe while gently agitating and remove from the water bath just before the last of the ice disappears.
2. Once only a small ice pellet remains, immediately remove the vial from the water bath, wipe dry, spray vial(s) with 70% ethanol and wipe dry again prior to placing i.
3. Immediately transfer the contents of the vial into 3 mL of warm Complete hRPTEC Culture Medium in a sterile 15 mL conical tube.
4. Rinse the vial with 1 mL of warm Complete hRPTEC Culture Medium and collect in the 15 mL tube.
5. Bring the volume to 15 mL with warm Complete hRPTEC Culture Medium.
6. Centrifuge 200 x g for 5 minutes at room temperature.
7. Aspirate and discard supernatant, leaving approximately 100 µL of medium covering the pellet.
8. Loosen the cell pellet by gently flicking the tube.
9. Re-suspend cells in 15 mL of Complete hRPTEC Culture Medium.
10. Add the hRPTEC suspension to the pre-warmed T75 flask.
11. Incubate overnight at 37°C and 5% CO_2.
12. Exchange with fresh warm Complete hRPTEC Culture Medium every other day until used for seeding in the chip.

Day -1: Chip Preparation

Goals:

- Activate the inner surface of the chip channels for proper ECM attachment
- Coat inner channels with a mixture of collagen IV and Matrigel® ECM proteins for cell attachment

Key Steps:

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

Required Materials:

- Chip-S1™ (12 chips per Zoë ™)
- ER-1 ™ reagent
- ER-2 ™ buffer
- 15 mL conical tubes
- DPBS (- / -) at room temperature
- DPBS (- / -) aliquot at 4°C
- Collagen IV (aliquot at 4°C on ice)
- Matrigel® (aliquot at 4°C on ice)
- 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, and all chips are in the same orientation. (See Figure 1)
3. Label each chip with ID numbers on the chip carrier tab.

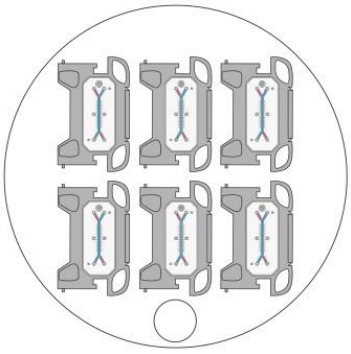


Figure 1: Proper orientation of Organ-Chips in 150 mm dish

Prepare ER-1™ Reagent

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 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™ Reagent to Channels

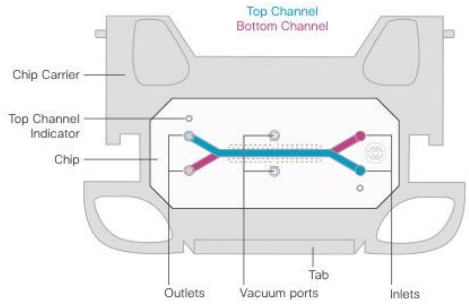


Figure 2: Top view of chip, with labelled ports, in chip carrier

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.
3. Without releasing the pipetting plunger, take the pipette out from the bottom channel inlet, and move the pipette containing 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.
5. Remove all excess ER-1 solution from the surface of the chip by gentle aspiration. Be sure only to remove ER-1 solution from the chip surface — do not aspirate ER-1 from the channels.
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.

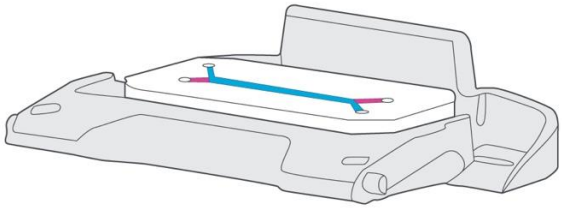


Figure 3: View of chip with no excess ER-1™ reagent on surface

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 15 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 Solution

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

For human Proximal Tubule Kidney-Chips, the ECM working concentration is:

Collagen IV: 50 µg / mL

Matrigel®: 100 µg / mL

1. Bring an ice bucket and ice to the BSC.
2. Thaw one aliquot of Collagen IV (1 mg / mL) 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.
    - Volume required per chip = approximately 100 µL
    - For every 12 chips to coat, prepare 1.5 mL of ECM solution (12 chips x 100 µL / chip + extra 300 µL = 1.5 mL of ECM solution). (See calculation example below.)
4. Combine components to prepare ECM working solution.
5. Keep the ECM solution on ice until ready to use.

Calculation Example

ECM Calculation Example ($C_1V_1 = C_2V_2$)

Collagen IV stock concentration: 1 mg / mL (C_1)

Collagen IV final concentration: 0.05 mg / mL (C_2)

Matrigel® stock concentration: 10 mg / mL (C_1)

Matrigel® final concentration: 0.1 mg / mL (C_2)

Stock volume: collagen IV or Matrigel® (V_1)

Final volume of ECM solution: 1.5 mL (V_2)

Collagen IV

(1 mg / mL) x (X mL) = (0.05 mg / mL) x (1.5 mL)

X = 75 µL of collagen IV stock solution

Matrigel®

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

Y = 15 µL of Matrigel® stock solution

DPBS

Volume DPBS =

(total volume of ECM needed) – (volume of collagen IV) – (volume of Matrigel®)

= 1500 µL – 75µL – 15 µL

= 1410 µL of DPBS

Coat Chips with ECM

1. Fully aspirate the cold DPBS from both channels.
2. Set a P200 pipette to take up 100 µL of ECM solution. (100 µL volume total will be used per chip.)
3. Carefully introduce ECM solution through the bottom channel inlet until a small ECM droplet forms on the outlet.
4. Without releasing the pipette plunger, take the pipette out from the bottom channel inlet, and move the pipette containing remaining ECM solution to the top channel inlet.
5. Introduce ECM solution through the top channel inlet, leaving small droplets of excess ECM solution on both ports in both channels. (See Figure 4 below.)
6. Inspect channels to ensure that no bubbles are present. If bubbles are present, dislodge by washing the channel with ECM solution until all bubbles have been removed.

7. Repeat steps 1 through 6 for each chip.
8. 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.

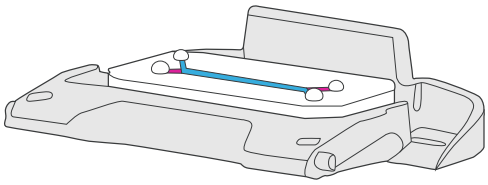


Figure 4: Chip in chip carrier with small droplets of ECM solution at ports

For best results, incubate the chips at 37°C overnight for seeding the following day. Note: If desired, cells can be seeded the same day as chip activation and ECM coating, after 4 hours post coating, though incubation overnight is preferred. If chips will be stored longer than overnight, store the chips at 4°C for up to 2 days, then incubate at 37˚C for 1 hour prior to seeding.

Day 0: hRMVECs and hRPTECs to Chip

Goals:

- HarvesthRMVECs and hRPTECs from flasks and seed in chip

Key Steps:

- Prepare and warm at 37°C Complete hRMVEC and hRPTEC Maintenance Media
- Prepare chips
- Harvest and count hRMVECs for seeding
- Seed hRMVECs to the bottom channel
- Harvest and count hRPTECs for seeding
- Seed hRPTECs to the top channel
- Gravity wash both channels with tips (3 hours post-seeding)

Required Materials:

- Complete hRMVEC Maintenance Medium (at 37°C)
- Complete hRPTEC Maintenance Medium (at 37°C)
- Serological pipettes
- Pipettes and filtered tips
- Aspirator and sterile tips
- 50 mL conical tubes
- Trypan blue counting solution
- Hemocytometer
- 70% ethanol
- Microscope

Prepare Complete hRMVEC and hRPTEC Maintenance Medium

The hRPTECs are seeded in the top channel in hRPTEC Maintenance Medium.

Complete hRMVEC Maintenance Media

Complete hRMVEC Maintenance Medium (50 mL)

Reagent	Volume	Conc. [Stock]	Conc. [Final]	Source	Cat. No.
Base RMVEC Culture Medium	49.75 mL	-	-	Recipe Above	-
FBS	0.25 mL	-	0.5%	Sigma	F4135

- Store at 4°C.
- Use within one week of preparation.

Complete hRPTEC Maintenance Media

Complete hRPTEC Maintenance Medium (50 mL)

Reagent	Volume	Conc. [Stock]	Conc. [Final]	Source	Cat. No.
Base RPTEC Culture Medium	49.75 mL	-	-	Recipe Above	-
FBS	0.25 mL	-	0.5%	Sigma	F4135

- Store at 4°C.
- FBS from the REGM™ SingleQuots™ Kitcan also be used to make this media.
- Use within a week of preparation.

Prepare Chips

1. Transfer ECM-coated chips from incubator into the BSC.
2. Fully aspirate ECM from both channels.
3. Pipette 200 µL of warm Complete hRMVEC Maintenance Medium to the bottom channel of each chip. Wash the channel by aspirating the outflow, while leaving media in the channel.
4. Pipette 200 µL of warm Complete hRPTEC Maintenance Medium to the top channel of each chip. Was the channel by aspirating the outflow, while leaving media in the channel.
5. Cover the 150 mm dish and return chips to the incubator until the cells are ready for seeding.

Harvest hRMVECs

hRMVECs in culture must be harvested and counted for bottom channel seeding. hRMVECs are adjusted to a density of 2 x 10^6 cells / mL prior to seeding the bottom channel. If the hRMVECs are not as proliferative as expected, the concentration can be increased up to 4 x 10^6 cells/mL in order to achieve a confluent monolayer within the channel.

1. Bring the culture flask containing hRMVECs from the incubator into the BSC.
2. Aspirate culture media and add 15 mL of 1X DPBS to wash the culture surface. Aspirate the DPBS wash.
3. Add 3 mL of trypsin-EDTA to the flask. 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 hRMVEC Maintenance Medium to the flask and pipette gently to mix, while collecting all cells from the culture surface.
6. Transfer the contents of the flask (12 mL) into a sterile 15 mL conical tube.
7. Add 3 mL of warm Complete hRMVEC Culture Medium to bring the total volume of the tube to 15 mL.
8. Centrifuge hRMVECs at 200 x g for 5 minutes at room temperature.
9. While the cells are in the centrifuge, prepare a Trypan Blue counting solution in a 1.5 mL tube:

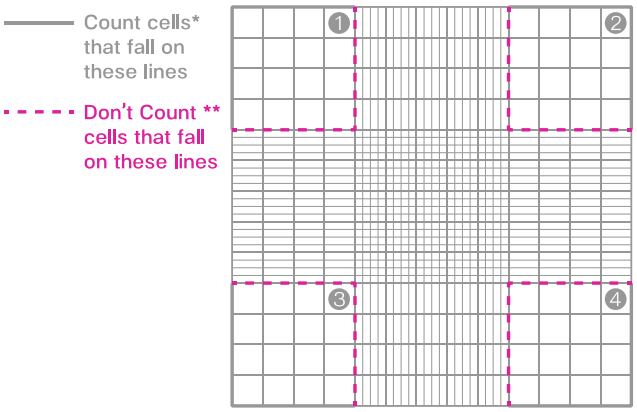
   40 µL Complete hRMVEC Maintenance Medium
   5 µL Trypan Blue

10. Carefully aspirate the supernatant, leaving approximately 100 µL of medium above the cell pellet. Note: The cell pellet will be very small. Aspirate carefully.
11. Loosen the cell pellet by flicking the tube gently.
12. Using a P1000 pipette, gently resuspend the cells by adding 400 µL of warm Complete hRMVEC Maintenance Medium.
13. Pipette gently to create a homogeneous mixture, and transfer 5 µL of the cell suspension to the Trypan Blue counting solution. (This will make a 1:10 dilution.)

Cell Counting and Viability Assessment

1. Count both viable and non-viable cells in each quadrant of the hemocytometer.
   Live Cell Count; Dead Cell Count; Total Cell Count
2. Calculate percent viability of the cell solution.
   (Live Cells) ÷ (Total Cells) x 100 = % Viability
3. 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)

4. Calculate viable cell yield.
   (Viable Cell Concentration) x (Cell Suspension Volume) = Viable Cell Yield (cells)



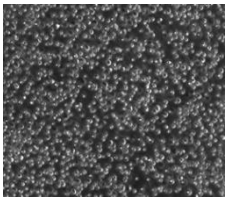
Dilute the hRMVECs with warm Complete hRMVEC Maintenance Medium to the required final cell density of 2.0 x 10^6 cells / mL.

Seed hRMVECs to Bottom Channel

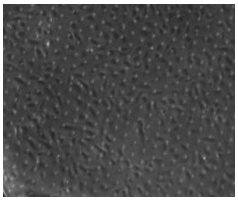
Work with one chip at a time. After seeding the first chip, assess cell density within the channel through the microscope, adjusting the density of the cell suspension accordingly for the subsequent chips if necessary.

1. Bring the 150 mm dish containing the 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 cell suspension before seeding each chip to ensure a homogeneous cell suspension.
4. Quickly and steadily pipette 15 to 20 µL of the cell suspension (at 2 x 10^6 cells / mL) into the bottom channel inlet port, while aspirating the outflow fluid from the chip surface. (Avoid direct contact with the outlet port.) Note: The rapid injection technique will provide homogeneous cell distribution throughout the culture area in the channel.
5. Cover the dish and transfer to the microscope to check the seeding density within the chip.
6. After seeding, invert each chip by resting the edge of the chip carrier on the chip cradle.

7. 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.
8. After confirming the correct cell density, seed cells in the remaining chips and flip the chips.
9. Place the chips (with the DPBS reservoir) at 37°C for 2.5 hours, or until cells have attached.
10. Once hRMVECs have attached (approximately 2.5 hours post-seeding), flip the chips back to upright position. Note: Remove the chip cradle and autoclave for use in next experiment.
11. With a P200 pipette, gently wash the bottom channel with 200 µL of media and return the chips to the incubator until ready to seed the hRPTECs in the top channel.



hRMVECs Seeding Density



hRMVECs 2.5 hours After Seeding

Seed a Conventional Well-Plate

It is recommended to always seed any remaining hRMVECs into a conventional well-plate as control for cell quality. If desired, transwells can be used as controls.

1. Once the chips have been seeded, dilute the remaining hRMVECs to a final cell density of 1.6 X 10^5 cells / mL in Complete hRMVEC Maintenance Medium.
2. Add 500µL of cell suspension to each well of a 24-well plate.
3. In the incubator, disperse the cells evenly across the bottom of the culture wells by moving the plate in a figure eight motion across the shelf at least 3 times, while keeping the plate flat on the surface of the incubator. Finally move the plate in a crisscross pattern to evenly disperse the cells at least 3 times. Once the cells are dispersed, do not disturb the plate until the next day to allow for cells to fully attach.

Harvest hRPTECs

Human RPTECs in culture must be harvested from the culture flask and counted for top channel seeding. HRPTECs are adjusted to a density of 1.0 x 10^6 cells / mL prior to seeding into the top channel of the Proximal Tubule Kidney-Chip.

1. Bring the culture flask containing hRPTECs from the incubator into the BSC.
2. Aspirate culture media and add 15 mL of 1X DPBS to wash the culture surface. Aspirate the DPBS wash.
3. Add 3 mL of trypsin-EDTA to the flask. 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 hRPTEC Maintenance Medium to the flask and pipette gently to mix, while collecting all cells from the culture surface.
6. Transfer the contents of the flask (12 mL) into a sterile 15 mL conical tube.
7. Add 3 mL of warm Complete hRPTEC Maintenance Medium to bring the total volume of the tube to 15 mL.
8. Centrifuge hRPTECs at 200 x g for 5 minutes at room temperature.
9. While the cells are in the centrifuge, prepare a Trypan Blue counting solution in a 1.5 mL tube:

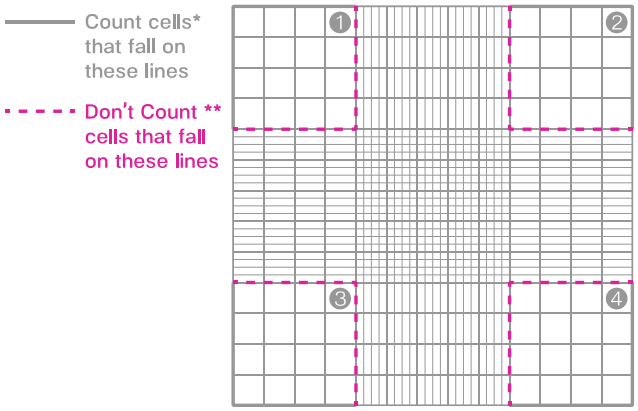
   40 µL Complete hRPTEC Maintenance Medium
   5 µL Trypan Blue

10. Carefully aspirate the supernatant, leaving approximately 100 µL of medium above the cell pellet. Note: The cell pellet will be very small. Aspirate carefully.
11. Loosen the cell pellet by flicking the tube gently.
12. Using a P1000 pipette, gently resuspend the cells by adding 400 µL of warm Complete hRPTEC Maintenance Medium.
13. Pipette gently to create a homogeneous mixture, and transfer 5 µL of the cell suspension to the Trypan Blue counting solution. (This will make a 1:10 dilution.)
14. Mix the counting solution thoroughly and count cells using a manual hemocytometer.

Cell Counting and Viability Assessment

1. Count both viable and non-viable cells in each quadrant of the hemocytometer.
   Live Cell Count; Dead Cell Count; Total Cell Count
2. Calculate percent viability of the cell solution.
   (Live Cells) ÷ (Total Cells) x 100 = % Viability
3. 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)

4. Calculate viable cell yield.
   (Viable Cell Concentration) x (Cell Suspension Volume) = Viable Cell Yield (cells)



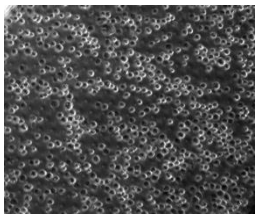
Dilute the hRPTECs with warm Complete hRPTEC Maintenance Medium to the required final cell density of 1.0 x 10^6 cells / mL.

Seed hRPTECs to Top Channel

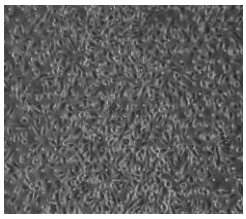
Work with one chip at a time. After seeding the first chip, assess cell density within the channel through the microscope. Adjust the density of cell suspension accordingly for the next chips if necessary

1. Bring the 150 mm dish containing the 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 cell suspension before seeding each chip to ensure a homogeneous cell suspension.
4. Quickly and steadily pipette 35 to 50 µL of the cell suspension (at 1.0 x 10^6 cells / mL) into the top channel inlet port, while aspirating the outflow fluid from the chip surface (avoid direct contact with the outlet port). Note: The rapid injection technique will provide homogeneous cell distribution throughout the culture area in the channel.
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 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.
8. Add DPBS in the cap of a 15mL conical tube and place it in the 150 mm culture dish that holds the chips.
9. Place the dish holding the chips at 37°C for 2.5 hours, or until cells have attached.



hRPTECs Seeding Density



hRPTECs 2.5 hours After Seeding

Seed a Well Plate

It is recommended to always seed any remaining hRPTECs into a plate as control for cell quality. If desired, transwells can be used as controls.

1. Once the chips have been seeded, dilute the remaining hRPTECs to a final cell density of 1.6 X 10^5 cells / mL in complete maintenance medium.
2. Add 500µL of cell suspension to each well of a 24-well plate.
3. In the incubator, disperse the cells evenly across the bottom of the culture wells by moving the plate in a figure eight motion across the shelf at least 3 times, while keeping the plate flat on the surface of the incubator. Finally move the plate in a crisscross pattern to evenly disperse the cells at least 3 times. Once the cells are dispersed, do not disturb the plate until the next day to allow for cells to fully attach.

Gravity Wash and Overnight Static Culture

A gentle gravity wash is performed after cells have fully attached (typically 2.5 to 3 hours post-seeding) 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 in the outlet. Because there are 2 different media being used, these two media must be separated by keeping them in filtered tips. Chips can be maintained overnight under static condition using pipette tips, as depicted below.

1. Gently pipette 200 µL of appropriate medium into the bottom channel inlet, until a small droplet appears on the outlet, or until a bubble is ejected through the outlet.
2. While the inlet has a pipette tip with medium, carefully place another fresh sterile 200 µL pipette tips in chip outlet port. Once you see the medium flow in the outlet tip, gently release the pipette tip in inlet port. Avoid pushing tips all the way down and release tip gently using the pipettor's tip injector — avoid forceful release so that tip doesn't block the channel. Repeat this for the top channel with warm Complete hRPTEC Maintenance Media.
3. Return chips with pipette tips inserted in each inlet and outlet port to the incubator overnight.
4. Maintain cells in static culture in chips until connecting to Pods and Zoë the next day.

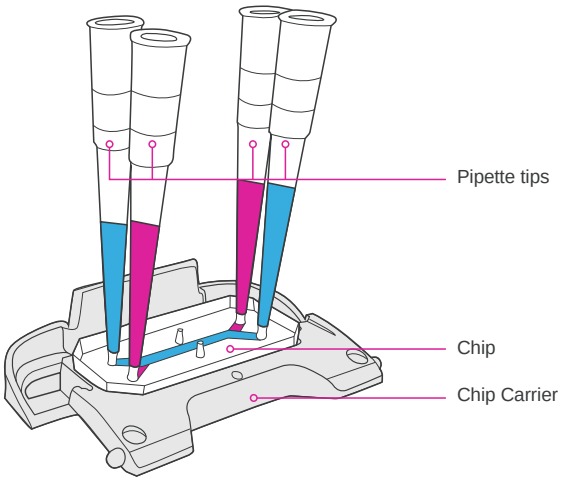


Figure 6: Chips with filtered tips inserted into ports with respective media

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
- Run Regulate cycle
- Begin experimental flow

Required Materials:

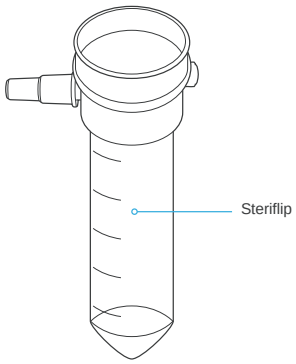
- Installed and qualified Zoë
- Prepared chips
- Pods (sterile) — 1 per chip
- Tray — 1 per 6 chips
- Steriflip® filtration unit: PVDF filter 0.45 µm (sterile)
- Vacuum source (minimum -70 kPa)
- Serological pipettes
- Pipettors and filtered tips
- 37°C water or bead bath
- 70% ethanol

Gas Equilibration of Media

The media equilibration step is 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 medium is outside — no more than 10 minutes — of a warmed environment (such as an incubator or bath), as gas equilibrium can become compromised when medium is allowed to cool.

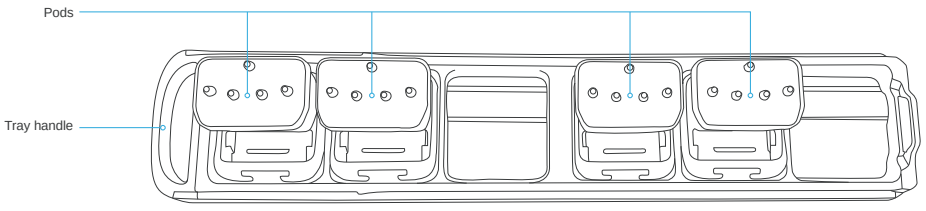
1. Place at least 3.3 mL of Complete hRPTEC Maintenance Medium for each chip in a 50 mL conical tube.
2. Place at least 3.3 mL of Complete hRMVEC Maintenance Medium for each chip in a separate 50 mL conical tube.
3. Warm both 50 mL conical tubes of media at 37°C in a water or bead bath for at least 1 hour.
4. 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 least -70 kPa. 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.
5. Remove the vacuum tubing from the Steriflip® units.
6. 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.

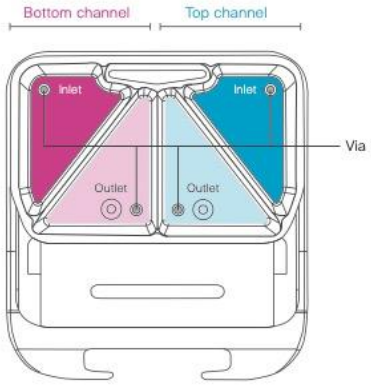
    As noted above, minimize the time media is outside of the incubator during Pod preparation to maintain temperature. This is an important step to ensure success of the chips.

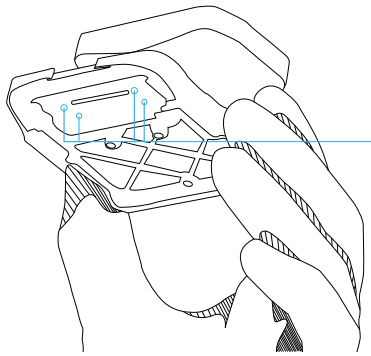


Prime Pods

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. Pipette 3 mL of pre-equilibrated, warm media to each inlet reservoir. In the top channel inlet reservoir add Complete hRPTEC Maintenance Medium; in the bottom channel inlet reservoir add hRMVEC Maintenance Medium.
4. Pipette 300 µL of pre-equilibrated, warm media to each outlet reservoir, 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 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.







Inspect underside of Pod to confirm that each port has a droplet of medium attached

Wash Chips

1. Transfer the seeded chips in a 150 mm dish from the incubator to the BSC.
2. Remove the pipette tips from the chip inlet and outlet ports.
3. Gently wash the top channel of each chip with warm, equilibrated Complete hRPTEC Maintenance Medium to remove any possible bubbles in the channel.
4. Place small droplets of equilibrated Complete hRPTEC Maintenance Medium on the top of each inlet and outlet port of each chip.
5. Gently wash the bottom channel of each chip with warm, equilibrated hRMVEC Maintenance Medium to remove any possible bubbles in channel.
6. Place small droplets of equilibrated hRMVEC Maintenance Medium on the bottom channel inlet and outlet ports of each chip.

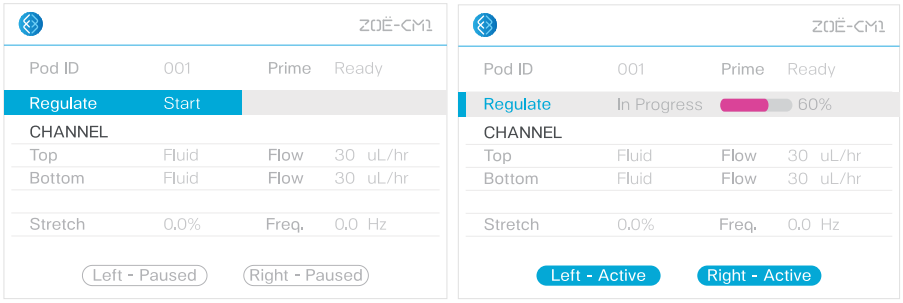
Chips to Pods

1. Holding one chip (while it remains in the chip carrier) in the dominant hand and one Pod 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 with connected the chip 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.

Pods to Zoë™

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 human co-culture Proximal Tubule Kidney-Chips, set the flow rate to 60 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.

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: Chip Maintenance and Sampling

Goals:

- Maintain Chips in Zoë™
- Cell culture inspection
- Collect samples for analysis

Key Steps:

- Maintenance and the Regulate cycle
- Sampling and media replenishment

Required Materials:

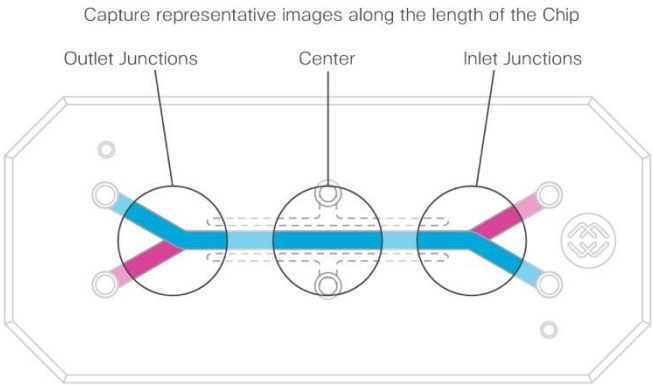
- Chips in Pods
- Cell culture media

Maintenance and the Regulate Cycle

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:
    1. 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.
    2. Repeat this wash step for each of the four Pod reservoirs.
4. Replace Pod lids and return the trays to Zoë.
5. Run the Regulate cycle again.
    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. At this point the “Activation” button will glow blue.
    4. The Regulate cycle lasts 2 hours. After the cycle completes, Zoë will begin flow at the preset Organ-Chip culture conditions.

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, avoiding 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:
    - 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.



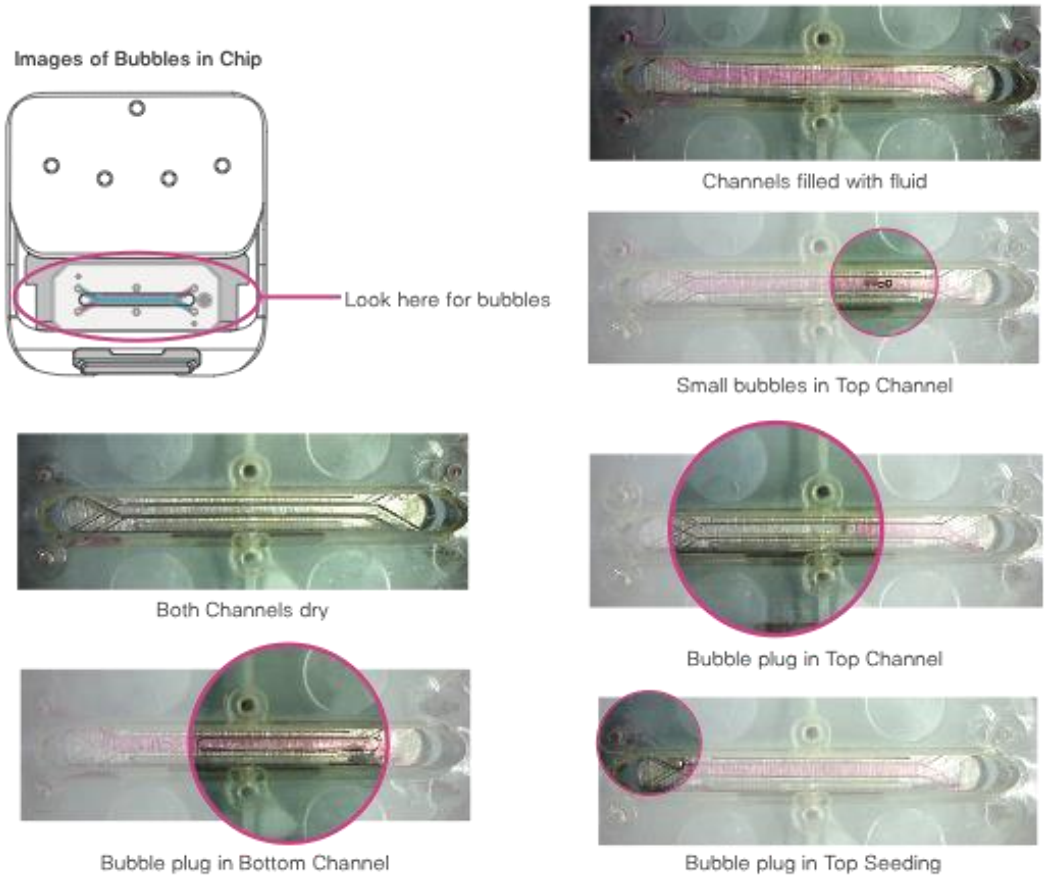
Troubleshooting

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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