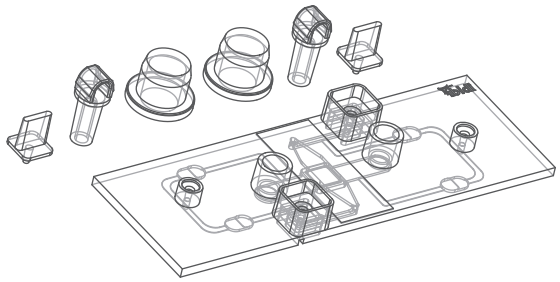
µ-Slide ibiPore SiN

Instruction Manual



The ibidi labware comprises a variety of µ-Slides, µ-Dishes, and µ-Plates, which have all been designed for high-end microscopic analysis of fixed or living cells. The high optical quality of the ibidi Polymer Coverslip is similar to that of glass, enabling a variety of recommended microscopy techniques with uncompromised resolution and choice of wavelength.

The µ-Slide ibiPore SiN consists of a horizontal porous membrane that is inserted between two channels. It is optimized for transmigration and transport studies in 2D or 3D under static or flow conditions with microscopic readout (e.g., endothelial barrier assays). The µ-Slide ibiPore SiN is available with different pore sizes, enabling assays with various cell types. It is not intended for transmembrane perfusion setups, filter assays, and applications with perfusion of both channels.

This document applies to the following products:

85216	**µ-Slide ibiPore SiN 0.5 µm / 20% ibiTreat**
85226	**µ-Slide ibiPore SiN 3 µm / 5% ibiTreat**
85236	**µ-Slide ibiPore SiN 5 µm / 5% ibiTreat**
85246	**µ-Slide ibiPore SiN 8 µm / 5% ibiTreat**

Shipping and Storage

This product is sterilized and sealed in a gas-permeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is outlined in the following table.

Conditions	
Shipping conditions	Ambient
Storage conditions	RT (15–25 °C)

Shelf Life	
ibiTreat	36 months

Material

The µ-Slide ibiPore SiN is made of a polymer that has the highest optical quality. The ibidi Polymer Coverslip bottom exhibits extremely low birefringence and autofluorescence, similar to that of glass. It is not possible to detach the bottom from the upper part. The slide is intended for one-time use and is not autoclavable, since it is only temperature-stable up to 80 °C/175 °F. Please note that gas exchange between the medium and the incubator's atmosphere occurs partially through the polymer coverslip, which should not be covered.

Optical Properties of Polymer Coverslip	
Refractive index (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 µm)
Material	Polymer

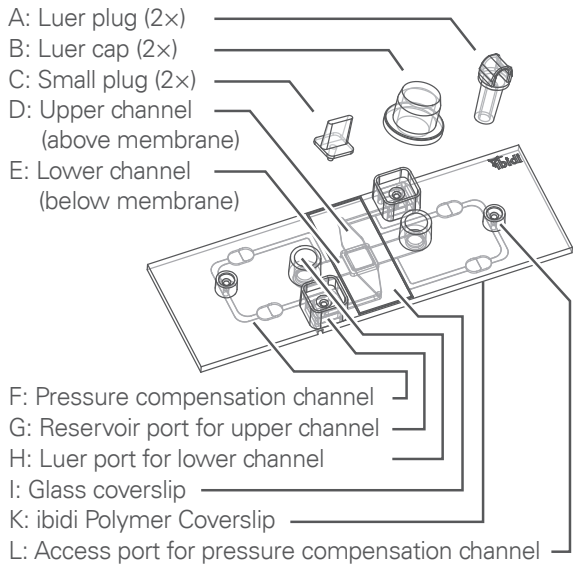
WARNING – The ibidi Polymer Coverslip is compatible with certain types of immersion oil only. A list of suitable oils can be found in the Section "Immersion Oil".

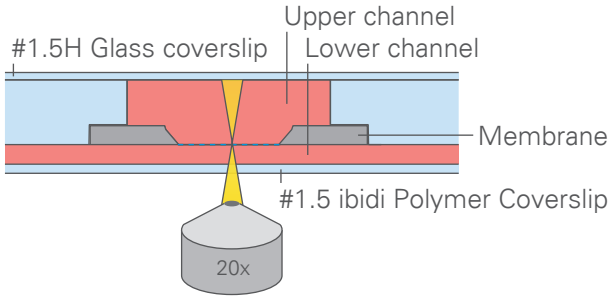
Surface

The µ-Slide ibiPore SiN is available with a porous membrane with four different pore sizes. The porous membrane is made of silicon nitride (SiN), a material with very high chemical and mechanical robustness. The 400 nm thick silicon nitride membrane is ideal for imaging and microscopy purposes, without any autofluorescence or transparency issues. The SiN material can be used directly for adherent cell culture or, optionally, it can be coated with extracellular matrix (ECM) proteins.

Geometry

The µ-Slide ibiPore SiN provides standard slide format according to ISO 8037/1.
It consists of a horizontal membrane that is inserted between two channels. The upper channel (D) is a static reservoir above the membrane. The lower channel (E) is a perfusion channel for applying defined shear stress on cells, which are attached to the membrane. The air-filled pressure compensation channel (F) buffers pressure changes, thus assuring convenient handling and membrane stability. The upper and the lower channel communicate with each other across the membrane only.





Cross-section of the µ-Slide ibiPore SiN.

Specifications of the µ-Slide ibiPore SiN				
Total coating area	4.50 cm^2			
Bottom	ibidi Polymer Coverslip			
Lower Channel (Main Channel)				
Access	Luer port, accessible with female Luers			
Volume	50 µl			
Height	0.4 mm			
Length	25 mm			
Width	5 mm			
Growth area	1.25 cm^2			
Upper Channel				
Access	Reservoir port, accessible with 20/200 µl pipet tips			
Volume	55 µl			
Height over membrane	1.3 mm			
Specifications of the Membrane				
Material	Silicon nitride (SiN)			
Thickness	400 nm			
Membrane size	2 mm × 2 mm			
Porous area	1.77 mm × 1.84 mm			
Restrictions for objective lenses	Working distance >0.5 mm			
Pore layout	Hexagonal spacing			
Available Variations				
Pore size (µm)	0.5	3	5	8
Porosity (%)	20	5	5	5
Pore-to-pore distance (µm)	1	12	20	32

Required Materials

The following materials are required for correctly using the µ-Slide ibiPore SiN.

- Sterile Petri dish (e.g., 10 cm)
- Sterile 1 ml syringe with male Luer tip
- Compatible, beveled 10–200 µl pipet tips:

Supplier	Ordering Number
Axygen	T-200-C, TR-222-C, TR-222-Y, or related tips
STARLAB	TipOne S1111-1816, S1111-1710, S1120-1840, or related tips
Sorenson BioScience	MultiFit Tip 10520, 10590, or related tips

Example of a compatible, beveled pipet tip.

General Handling

CAUTION – Strictly follow the instructions in this document. All steps are essential for successfully handling the µ-Slide ibiPore SiN. Do not change the order of pipetting, volumes, or plug positions. Modifications in the protocol might lead to air bubbles, cell damage, or membrane disruption.

- Always handle the µ-Slide ibiPore SiN carefully because the porous membrane can be easily damaged by abrupt pressure changes.
- Always use caution when connecting plugs, tubing, and pump connectors.
- Before starting an experiment, check the integrity of the membrane microscopically.
- The µ-Slide ibiPore SiN should not be placed directly on cold or metal surfaces, as this can cause rapid cooldown of medium and cells. Fast temperature changes promote the formation of air bubbles and cell stress, resulting in cell detachment and apoptosis. Always place the µ-Slide ibiPore SiN on a µ-Slide Rack (ibidi, 80003) or inside a sterile 10 cm Petri dish.
- Use sterile-filtered medium to avoid optical impairment of the membrane, which are caused by non-soluble components of the culture medium or serum.
- The µ-Slide ibiPore SiN is intended for perfusion and shear stress applications with a connected pump. Static assays are possible but will require frequent medium exchange or persistent, gentle flow. The latter can also be achieved using an incubator-compatible cell culture rocker.

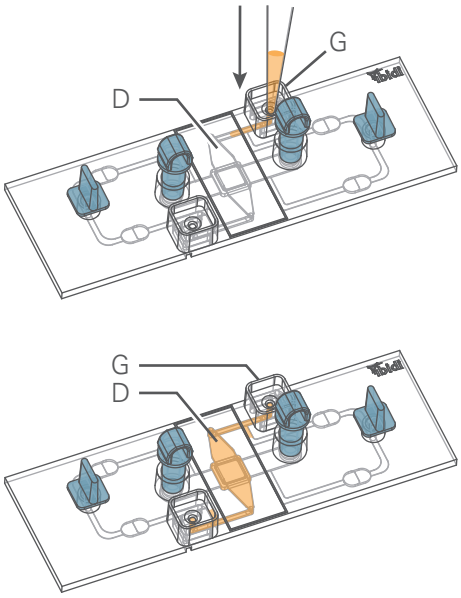
TIP – The final amount of liquid and air in the pressure compensation channel is flexible and depends on the pressure conditions. The volume of air inside the pressure compensation channel can be adjusted by opening the access port (L) and changing the air volume with a pipet. Ensure that both the upper and lower channels are filled with liquid and disconnected from any tubing during this air volume adjustment step.

TIP – The day before seeding the cells, we recommend placing the cell medium, the slide, and possible tubing for perfusion into the incubator for equilibration. This will prevent the liquid inside the channel from forming air bubbles during the incubation time. The packaging of the µ-Slide is made of a gas-permeable material, therefore unpacking is not necessary for the gas equilibration step.

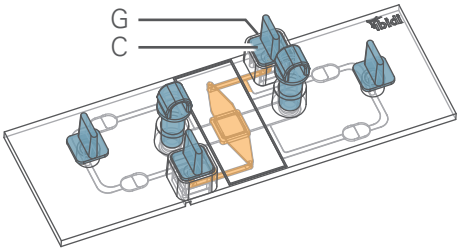
Coating

The following protocol provides a procedure for coating the µ-Slide ibiPore SiN. Most cells, particularly primary cells, require ECM proteins, such as collagen or fibronectin, to promote cell adherence and growth on the porous membrane.
Please also read Application Note 08: Coating Protocols for ibidi Labware.

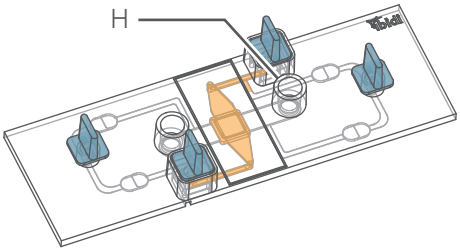
1. Close both Luer ports (H) of the lower channel (E) with the Luer plugs (A).
2. Close the access ports (I) of both pressure compensation channels (F) with the small plugs (C).
3. Prepare your coating solution according to the manufacturer's specifications. Adjust the concentration to a coating area of 4.5 cm^2 and a volume of 105 µl per channel. Prepare ca. 350 µl coating solution per slide.
4. Fill 55 µl of the coating solution into the upper channel (D) using the reservoir port (G). Use a standard pipet with the recommended pipet tips.



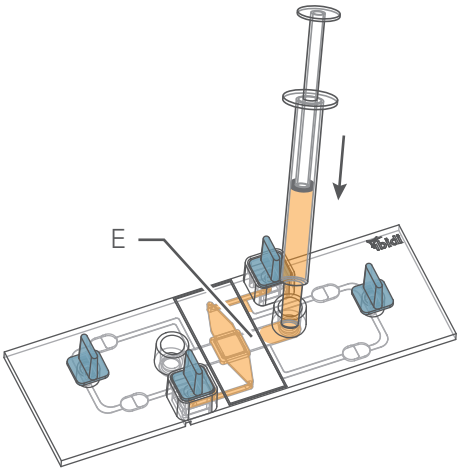
5. Close both reservoir ports (G) with the small plugs (C).



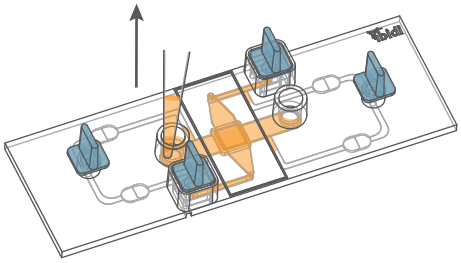
6. Remove the Luer plugs (A) from the Luer ports (H).



7. Apply ca. 150 µl of the coating solution into the lower channel (E) using a biocompatible 1 ml syringe filled with ca. 300 µl solution. Typically, a standard pipet provides insufficient force to fill the entire lower channel conveniently without any air bubbles.



8. Remove any leftover coating solution from the Luer ports with a standard pipet. Take care not to remove any solution from the channel by holding the pipet tip away from the channel in the Luer port.



9. Put the slide into a sterile 10 cm Petri dish and leave at room temperature for at least 30 minutes.

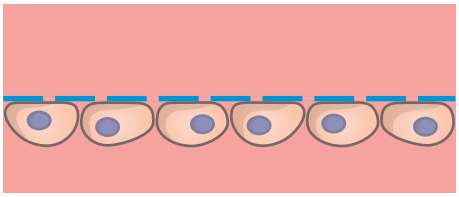
10. Wash both the upper and the lower channel with PBS or medium. To do this, follow the protocols in the Sections “Medium Exchange in the Upper Channel” (page 9) and “Medium Exchange in the Lower Channel” (page 10).

11. Aspirate the remaining solution completely from both the upper and the lower channel.

12. Let the slide dry at room temperature.

CAUTION – Before continuing with the experiment after coating and washing, all channels must be completely dry.

Seeding Cells on the Membrane’s Lower Side

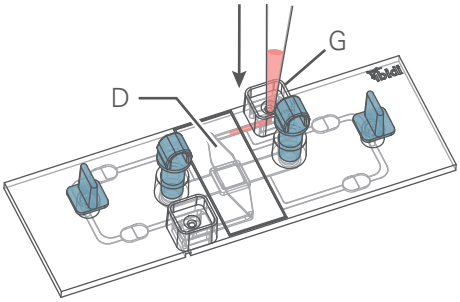


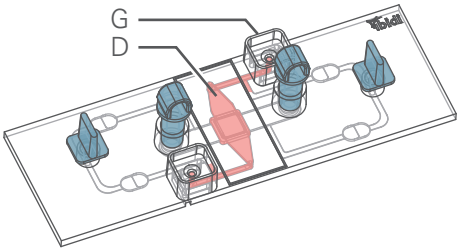
To perform assays with cells on the lower side of the membrane, use the steps outlined in the following protocol.

1. Close both Luer ports (H) of the lower channel (E) with the Luer plugs (A).

2. Close the access ports (I) of both pressure compensation channels (F) with the small plugs (C).

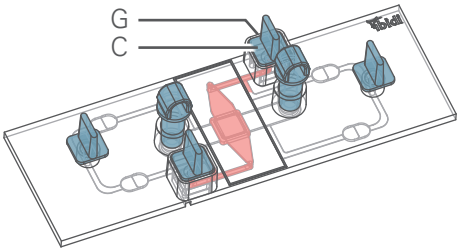
3. Fill the upper channel (D) by gently injecting 55 µl cell-free medium into one of the reservoir ports (G). Press the pipet tip into the reservoir port (G) and hold the pipet upright to create a seal. Placing the liquid onto the opening without injection will not properly fill the channel. Inject medium until the entire channel is filled.





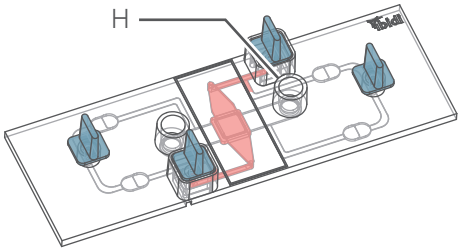
CAUTION – Fill the upper channel only once. Refilling the upper channel may lead to a trapped air bubble. For a medium exchange in the upper channel, follow the protocol in the Section “Medium Exchange in the Upper Channel” (page 9).

4. Close both reservoir ports (G) with the small plugs (C).

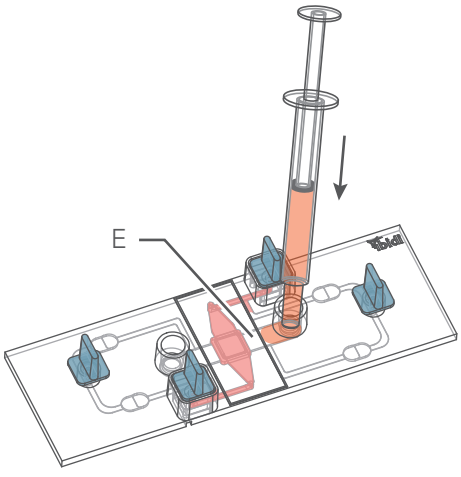


5. Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 3–7 × 10^5 cells/ml suspension should result in a confluent layer within 2–3 days. For endothelial cells under flow conditions, we recommend a high concentration of 1.2–2.5 × 10^6 cells/ml for 100% optical confluency after cell attachment.

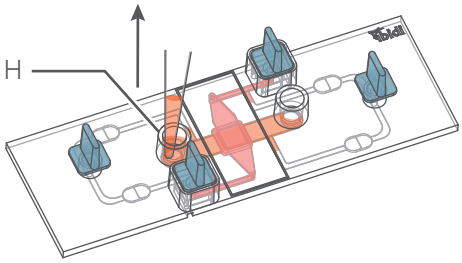
6. Remove the Luer plugs (A) from the Luer ports (H).



7. Apply ca. 150 µl cell suspension into the lower channel (E) using a biocompatible 1 ml syringe filled with ca. 300 µl cell suspension. Typically, a standard pipet provides insufficient force to fill the entire lower channel conveniently without any air bubbles.



8. Completely remove the cell suspension from the Luer ports (H) with a standard pipet tip. Take care not to remove any cell suspension from the channel by holding the pipet tip away from the channel in the Luer port.

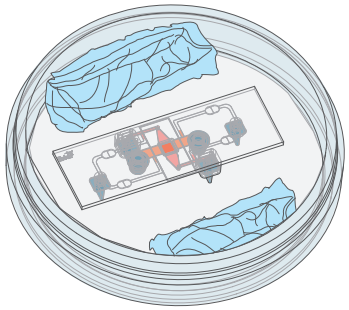


9. Cover the Luer ports (H) with the Luer caps (B). Do not use the Luer plugs (A) in this step.

10. Put the slide into a sterile 10 cm Petri dish with wet tissue. This ensures high humidity and low evaporation during cell attachment.

11. Close the Petri dish.

12. Turn the Petri dish upside down in order to let the cells attach downside of the membrane. Without this rotation step, the cells will not attach on the membrane but rather to the bottom of the channel.

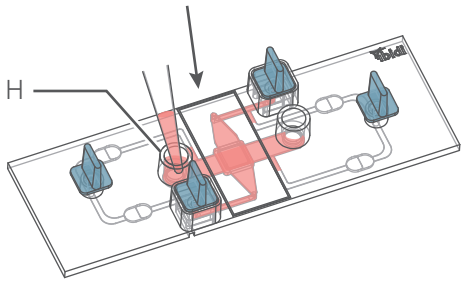


13. Put the Petri dish into the incubator for cell adhesion. Incubate as usual (e.g., at 37 °C and 5% CO_2).

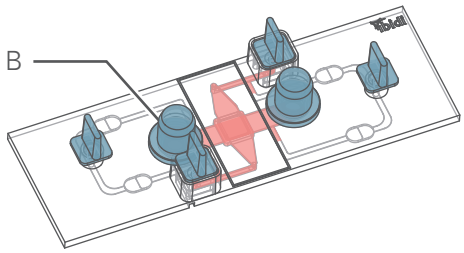
14. Observe the cell attachment under the phase contrast microscope leaving the slide inside the Petri dish. When cells are attached, continue with the protocol.

15. Turn the Petri dish to bring it into the standard position again.

16. Fill each Luer port (H) with 60 µl cell-free medium. For flow applications, fill both Luer ports (H) until they are completely filled (80 µl). This ensures air bubble-free connection of the tubing. Continue with the Section “Connecting Tubing for Perfusion” (page 8) for connecting tubing and pumps.



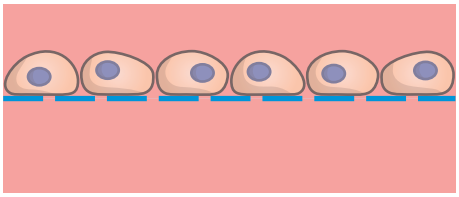
17. Cover the Luer ports (H) with the Luer caps (B) to maintain sterility.



18. Conduct your experiment.

TIP – For longer cultivation under static conditions, exchange the medium in the upper and/or lower channel at regular intervals. Depending on the number of cells and medium consumption, we recommend a medium exchange every 1–2 days.

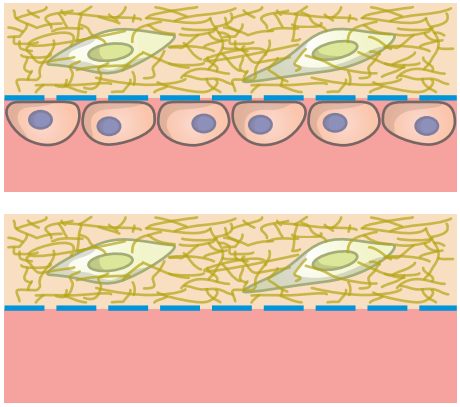
Seeding Cells on the Membrane's Upper Side



To perform assays with cells on the upper side of the membrane, follow all steps and the order of pipetting as described in the Section "Seeding Cells on the Membrane's Lower Side" on page 5, but change the following steps:

- Steps 3: Instead of using cell-free medium, fill the upper channel with cell suspension. Before filling the channel, trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 0.9–2.1 × 10^5 cells/ml suspension should result in a confluent layer within 2–3 days. For 100% optical confluency directly after cell attachment, we recommend a concentration of 3.5–7.5 × 10^5 cells/ml. Step 3 covers the actions outlined in Step 5.
- Step 7: Fill the lower channel with cell-free culture medium.
- Skip steps 12 and 15. The Petri dish can be cultivated in the standard position.

Preparing a Gel Matrix Inside the Upper Channel



To perform assays with a gel matrix inside the upper channel, follow all steps and the order of pipetting as described in the Section "Seeding Cells on the Membrane's Lower Side" on page 5, but change the following steps:

- Step 3: For filling the upper channel, use a liquid gel matrix, optionally mixed with cells. For this, prepare your gel matrix according to the manufacturer's specifications and, optionally, add your cell suspension at the desired concentration. Then, continue as described in step 3. Finally, let the gel polymerize as described in the manufacturer's specifications. Step 3 covers the actions outlined in Step 5.

- Step 7: Fill the lower channel with cell-free culture medium. Alternatively, use a cell suspension to attach a cell monolayer to the membrane's lower side.

CAUTION – Please keep in mind that medium exchange is not possible in a channel filled with a gel matrix.

Connecting Tubing for Perfusion

For shear stress and flow applications, the lower channel of the µ-Slide ibiPore SiN can be perfused. The slide is fully compatible with the ibidi Pump System and other pump setups. Please note that the upper channel cannot be perfused with any liquids.

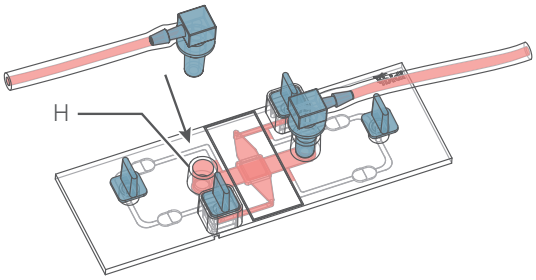
CAUTION – Applying liquid perfusion to the upper channel is not recommended due to varying channel heights, which create different shear stress conditions in the upper channel. In contrast, liquid flow and shear stress in the lower channel are homogeneous across the channel's and membrane's surface.

Detailed information about flow rates, shear stress, and shear rates is provided in Application Note 11: Shear Stress and Shear Rates for ibidi µ-Slides Based on Numerical Calculations. We provide suitable Tube Adapter Sets (ibidi, 10831). These sets include a 20 cm tubing with an inner diameter of 1.6 mm and adapters for connecting the µ-Slide (female Luer) to the tubing of the used pump.

1. Prepare the perfusion system by first filling the tubing completely with medium and then pinching off the tubing with the ibidi Screw Clamp (ibidi, 10861) or a similar Hoffmann tubing clamp.

CAUTION – Always use a screw clamp or a similar Hoffmann tubing clamp, which releases the pressure slowly. Do not use quick-release pinching clamps such as the ibidi Hose Clip (ibidi, 10821). Those can cause pressure peaks leading to cell and membrane damage.

2. Connect the male Luer ends of the clamped tubing to the Luer ports (H) one at a time. Make sure not to trap air. The correct and air-bubble free flow connection will cause minor medium spillage, which can be removed with a tissue.



3. Open the clamped tubing slowly by gradually unscrewing the clamp. This is essential to minimize pressure peaks within the channel.

4. Conduct your perfusion experiment leaving all small plugs (C) in place. Ensure they form a tight seal to prevent leakage.

TIP – When the µ-Slide connected to the tubing is placed in the incubator, you can put it into a tray (e.g., a Petri dish). That keeps the incubator clean in case of any leakage.

Shear Stress Calculations

To calculate the shear stress (τ) in the µ-Slide ibiPore SiN, insert the flow rate (Φ) and the dynamic viscosity (η) in the formulas provided below:

$$\tau = \eta \cdot 131.6 \cdot \Phi$$

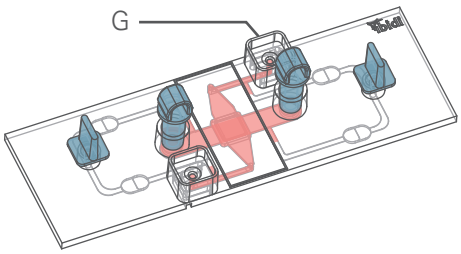
For simplicity, the calculations include conversions of units (not shown). Please insert the values in the unit definitions given below:

Shear stress	$\tau \left[\frac{dyn}{cm^2}\right]$
Dynamical viscosity	$\eta \left[\frac{dyn \cdot s}{cm^2}\right]$
Flow rate	$\Phi \left[\frac{ml}{min}\right]$

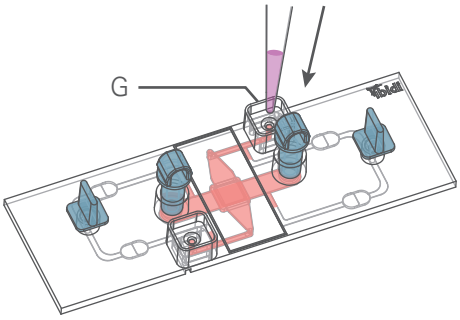
Medium Exchange in the Upper Channel

CAUTION – Take care that the channels never fall dry during the exchange process. This helps avoid air bubbles.

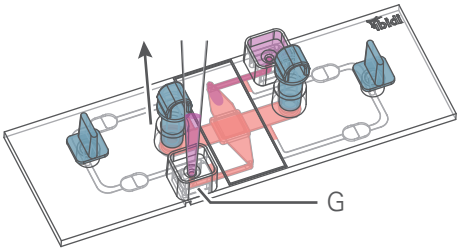
1. If the lower channel (E) is connected to a tubing, pinch off the tubing on both sides of the channel with the ibidi Screw Clamp (ibidi, 10861) or a similar Hoffmann tubing clamp. The tubing can stay connected to the Luer ports. If no tubing is connected, close both Luer ports (H) of the lower channel (E) with the Luer plugs (A).
2. Remove the small plugs (C) from the reservoir ports (G).

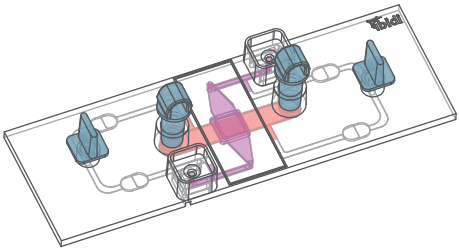


3. Put 60 µl new culture medium on top of one reservoir port (G) of the upper channel. Do not inject directly. Make sure not to trap or inject any air.



4. Slowly remove 60 µl from the opposite reservoir port (G). To create a seal, press the pipet tip upright into the reservoir port while aspirating. This will replace the liquid in the upper channel.



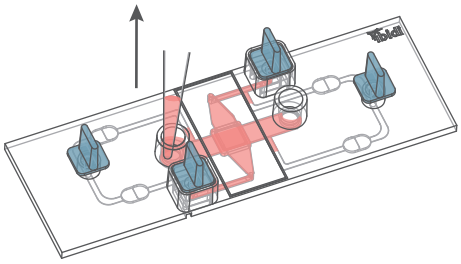


5. Repeat the medium exchange if necessary.
6. Close both reservoir ports (G) with the small plugs (C).
7. Reconnect any tubing and continue with your experiment.

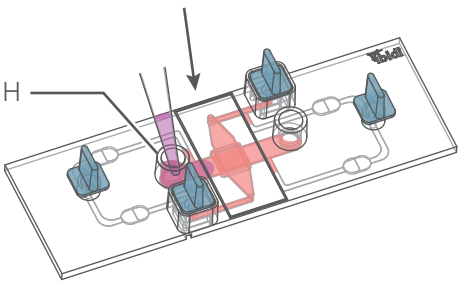
Medium Exchange in the Lower Channel

CAUTION – Take care that the channel never falls dry during the exchange process. This helps avoid air bubbles.

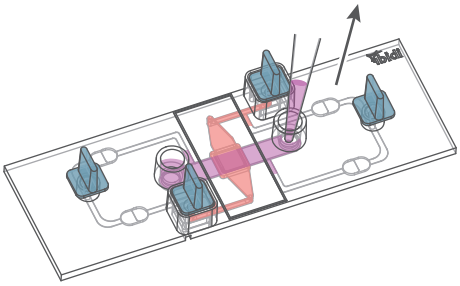
1. If the lower channel (E) is connected to a tubing, pinch off the tubing on both sides of the channel using the ibidi Screw Clamp (ibidi, 10861) or a similar Hoffmann tubing clamp.
2. Make sure the reservoir ports (G) are closed with the small plugs (C).
3. Disconnect the tubing from the Luer ports (H) of the lower channel (E).
4. Remove all liquid from the Luer ports (H) with a standard pipet tip. Take care not to remove any liquid from the channel by holding the pipet tip away from the channel in the Luer port.



5. Apply 100 µl new culture medium into one of the Luer ports (H).



6. Slowly remove 100 µl from the opposite Luer port (H), pointing the pipet tip towards the channel inlet to connect with the liquid. This will replace the liquid in the lower channel.



7. Repeat the medium exchange if necessary.
8. Fill each Luer port (H) with 60 µl culture medium. Fill both Luer ports (H) until they are completely filled (80 µl). This ensures air bubble-free connection of the tubing.
9. Continue with your experiment.

Chemical Compatibility

The following table provides some basic information on the chemical and solvent compatibility of the µ-Slide ibiPore SiN. For a full list of compatible solvents and more information on chemical compatibility, visit ibidi.com/chemicals.

Chemical / Solvent	Compatibility
Methanol	Yes
Ethanol	Yes
Formaldehyde	Yes
Acetone	Yes, without lid
Mineral oil	No
Silicone oil	Yes
Immersion oil	See Section “Immersion Oil”

Microscopy

To image your cells, no special preparations are necessary. Living or fixed cells can be directly observed, preferably on an inverted microscope. The bottom cannot be removed. For optimal results in fluorescence microscopy and for storage of fixed and stained samples, ibidi provides mounting media that are optimized for ibidi labware:
Cat. No. 50001: ibidi Mounting Medium
Cat. No. 50011: ibidi Mounting Medium with DAPI

CAUTION – Due to the channel height of 0.4 mm, the membrane can be imaged only with objective lenses that have a working distance larger than 0.5 mm.

CAUTION – Please keep in mind that the 0.5 µm pores are not visible when using a low-resolution cell culture microscope.

Immersion Oil

WARNING – When using oil immersion objectives with the ibidi Polymer Coverslip, use only the immersion oils specified in the table below. The use of any non-recommended oil could damage the ibidi Polymer Coverslip. The resulting leakage may harm objectives and microscope components. All immersion oils that are not listed in the table below should be considered non-compatible.

Company	Product	Ordering No.	Lot Number	Test Date
ibidi	ibidi Immersion Oil 2	50102	24-07-04	07/2024
Cargille	Type A	16482	100592	01/2017
Cargille	Type HF	16245	92192	01/2017
Carl Roth	Immersion oil	X899.1	414220338	01/2017
Leica	Immersion Liquid	11513859	n.a.	03/2023
Leica	Immersion Liquid Type G	11513910	n.a.	04/2024
Nikon	Immersion Oil F2 30cc	MXA22192	n.a.	01/2020
Nikon	Silicone Immersion Oil 30cc	MXA22179	20191101	01/2020
Olympus	Silicone Immersion Oil	SIL300CS-30CC	N4190800	01/2017
Zeiss	Immersol 518 F	444960-0000	220211	03/2023
Zeiss	Immersol 518 F (30 °C)	444970-9010	220816	03/2023
Zeiss	Immersol 518 F (37 °C)	444970-9000	220302	03/2023
Zeiss	Immersol W 2010	444969-0000	101122	04/2012
Zeiss	Immersol Sil 406	444971-9000	80730	03/2023
Zeiss	Immersol G	462959-9901	211117	03/2023

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Further information can be found at ibidi.com. For questions and suggestions, please contact us by e-mail at info@ibidi.com or by telephone at +49 (0)89/520 4617 0.