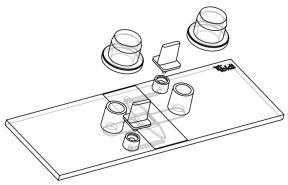


The ibidi product family is comprised of a variety of µ–Slides and µ–Dishes, which have all been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength.
The µ–Slide Membrane ibiPore Flow allows you to perform several cell culture assays with an optical porous glass membrane.

The main applications are trans–endothelial migration under flow conditions, co–cultivation and transport studies in 2D and 3D, polarity assays, and skin and lung models with epithelial cells.

Material

ibidi µ–Slides, µ–Dishes, and µ–Plates are made of a plastic that has the highest optical quality. The polymer coverslip on the bottom exhibits extremely low birefringence and autofluorescence, similar to that of glass. Also, it is not possible to detach the bottom from the upper part. The µ–Slides, µ–Dishes, and µ–Plates are not autoclavable, since they are only temperature–stable up to 80°C/175°F. Please note that gas exchange between the medium and incubator's atmosphere occurs partially through the polymer coverslip, which should not be covered.

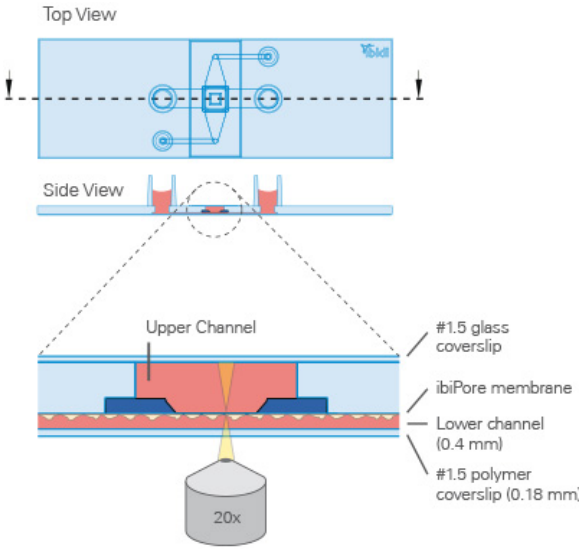
Optical Properties ibidi Polymer Coverslip	
Refractive index n_D (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 µm)
Material	polymer coverslip

Please note! The ibidi polymer coverslip is compatible with certain types of immersion oil only. A list of suitable oils can be found on page 6.

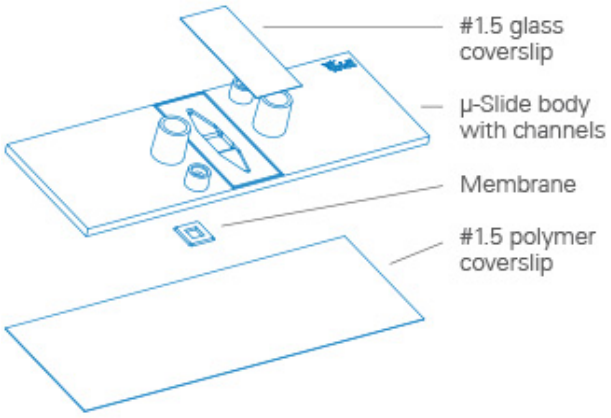
Geometry

The µ–Slide Membrane ibiPore Flow provides standard slide format according to ISO 8037/1. The principle of the µ–Slide consists of a horizontal membrane inserted between two chambers. Two channels, each with an inlet and outlet port are feeding the chambers. The main channel is connected to the lower chamber. The upper channel to the chamber above the membrane. The channels do only communicate with each other across the membrane.

Geometry of the µ–Slide Membrane ibiPore Flow	
Total coating area	4.50 cm^2
Bottom	ibidi Standard Bottom
Main Channel (Lower Channel)	
Adapters	Female Luer
Volume	50 µl
Height	0.4 mm
Length	25 mm
Width	5 mm
Growth area	1.25 cm^2
Upper Channel	
Adapters	ibidi Adapter for 20/200 µl pipet tips
Volume	55 µl
Height	various
Height over membrane	1.3 mm



Cross section of the µ–Slide Membrane ibiPore Flow



Parts of the µ–Slide Membrane ibiPore Flow

Geometry of the Membrane	
Material	SiO_2 (glass)
Thickness	0.3 µm (300 nm)
Membrane size	2 mm × 2 mm
Restrictions for objective lenses	Working distance >0.5 mm
Maximum pressure across the membrane	100 mbar
Pore layout	Hexagonal spacing

Available variations				
Pore Size	0.5 µm	3 µm	5 µm	8 µm
Porosity	20%	5%	5%	5%
Pore-to-pore distance	1 µm	12 µm	20 µm	32 µm

Shipping and Storage

The µ–Slide Membrane ibiPore Flow is sterilized and welded in a gas-permeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is listed in the following table.

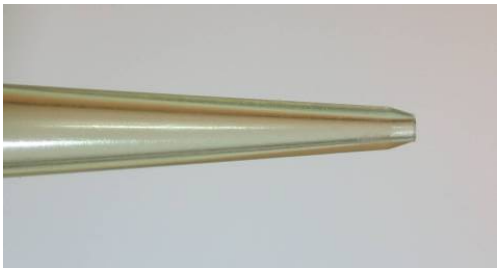
Conditions	
Shipping conditions	Ambient
Storage conditions	RT (15-25°C)
Shelf life	36 months

Handling

Always handle the µ–Slide Membrane ibiPore Flow with care. Since the SiO_2–membrane is very fragile, any abrupt movement should be avoided, especially after closing the upper channel by using the plugs.

The following 10-200 µl pipet tips for filling the upper channel are recommended:

Supplier	Type of Pipet Tip
Greiner Bio-One	739261, 739280, 739290, 772288 or related beveled Greiner tips
Axygen	T-200-C, TR-222-C, TR-222-Y or related Axygen beveled tips
STARLAB	TipOne RPT S1161-1800 or related beveled TipOne tips
Sorenson BioScience	MulTi Fit Tip 10590, 15320, 15330 or related beveled Sorenson BioScience tips



Beveled Pipet Tip, see list for correct models.

Important!

Strong flow across the membrane must be avoided by very gentle plug handling and filling the cell–free side of the membrane first. Adherent cells on the membrane might be damaged due to the potential flow across the membrane. To avoid this strictly follow the cell seeding protocol in this document.

Important!

Always use sterile–filtered medium to avoid optical impairment of the membrane caused by non–soluble components of the culture medium or serum.

Surfaces

The µ–Slide Membrane ibiPore Flow is available with the ibiTreat surface. ibiTreat is a physical treatment and optimized for adhesion of most cell types. Many cell lines as well as primary cells were tested for good cell growth. The porous membrane is an uncoated glass membrane. Protein

coatings increase direct cell growth of adherent cells on the glass membrane.

Coating

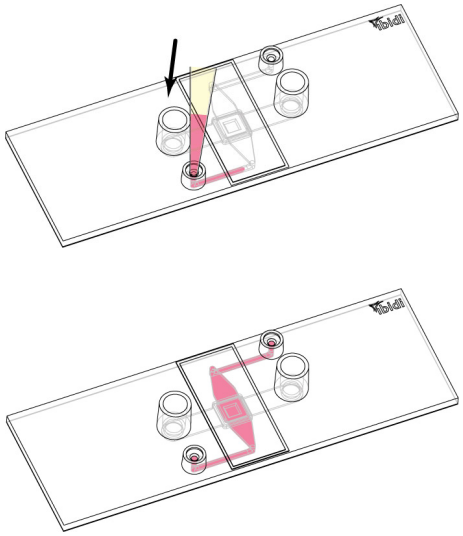
Specific coatings are possible following this protocol:

- Prepare your coating solution according to the manufacturer's specifications or reference. Adjust the concentration to a coating area of 4.5 cm^2 and a volume of 105 µl.
- Fill 55 µl of the coating solution into the upper channel using a standard pipet and the recommended pipet tips.
- Apply 50 µl of the coating solution into the lower channel using a 1 ml syringe.
- Put into a sterile 10 cm Petri dish and leave at room temperature for at least 30 minutes.
- Aspirate the solution and wash with PBS or medium. Let dry at room temperature.

Seeding Cells on the Membrane's Lower Side

It is possible to cultivate cells on both sides of the membrane. When seeding cells on the lower side of the membrane, always fill the upper channel with medium before seeding the cells! To seed cells on the lower side of the Membrane follow these steps:

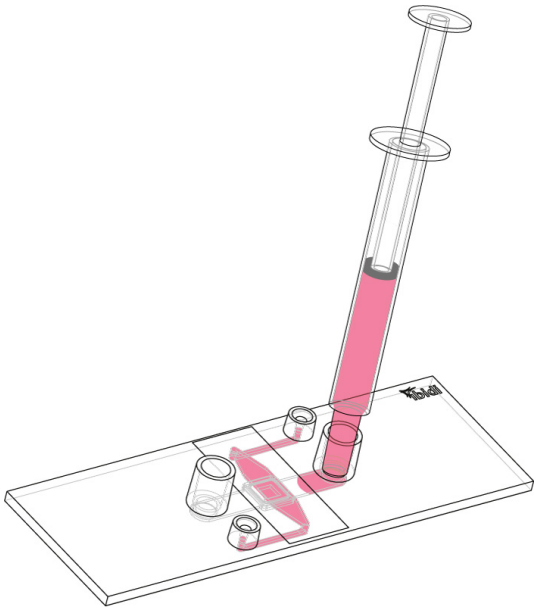
- Fill the upper channel with 55 µl cell–free medium. Inject the liquid directly into the injection port.



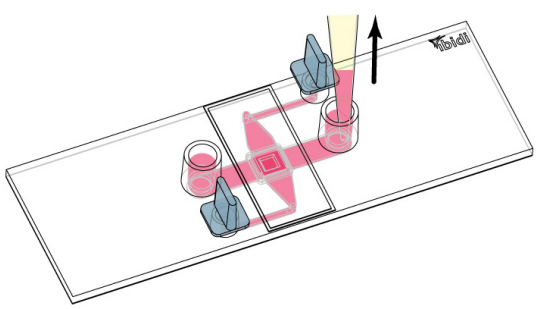
Important!

Fill the upper channel only one time. Re–filling the upper channel may lead to a trapped air bubble. For a medium exchange in the upper channel, follow the protocol on page 4.

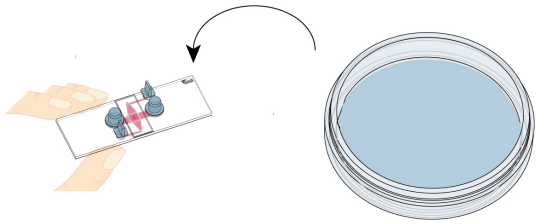
- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 3-7 × 10^5 cells/ml suspension should result in a confluent layer within 2-3 days. For endothelial cells under flow conditions we recommend a high concentration of 1.2-2.5 × 10^6 cells/ml for 100% optical confluency after cell attachment.
- Apply ca. 150 µl cell suspension into the lower channel by using a 1 ml syringe.



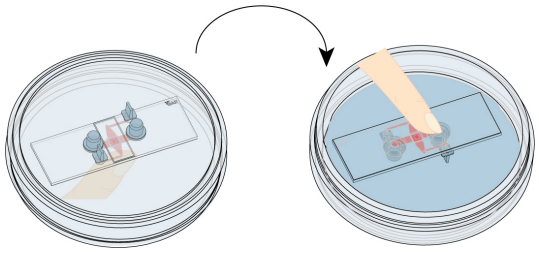
- Close the upper channel by using the plugs. Take care to push the plugs gently into the injection ports!
- Remove leftover cell suspension from the reservoirs.



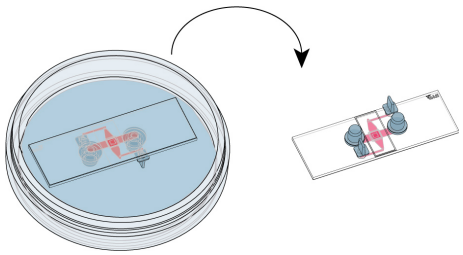
- Cover the reservoirs with the supplied lids and turn upside down: Use a 10 cm Petri dish and place it upon the µ–Slide.



- Turn around both, the µ–Slide and the Petri dish.



- Incubate at 37°C and 5% CO_2 as usual. Await cell attachment in order not to flush out the cells.
- After cell attachment, turn around the µ–Slide again.



- Fill each reservoir with 60 µl cell–free medium for longer cell cultivation under static conditions. For flow applications, fill the reservoir until it is full and connect a Luer adapter and tubing afterwards.

Tip!

The day before seeding the cells we recommend placing the cell medium and the µ–Slide into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles over the incubation time.

Important!

When seeding cells, fill only the correct channel volume into the channel. Avoid surplus cell suspension in the reservoirs.

Medium Exchange

Exchanging medium in the upper channel

Put 15 µl new solution on top of one adapter of the upper channel. Do not inject directly and don't trap air bubbles. Immediately after this, slowly remove 15 µl from the opposite side. Repeat this step 10 times for a 99% medium exchange.

Exchanging medium in the lower channel

Empty the reservoirs completely without emptying the channel. Inject the new solution from one side and remove the old solution from the other side. For a 99% exchange add 150 µl from one side and remove 150 µl from the opposite side.

Important!

Take care, that the channel never falls dry during the exchange process. This helps you avoiding air bubbles!

Adapting Tubes to the Upper Channel

For gas flow application to the upper channel a 6 mm long piece of silicone tubing may be used. After putting the silicone tubing over the pipet adapter it will act as a Female Luer adapter to support Male Luer adapters. We recommend a Tygon 3350 silicone tubing (Saint Gobain Performance Plastics) with 3.2 mm inner diameter and 4.8 mm outer diameter.

Important!

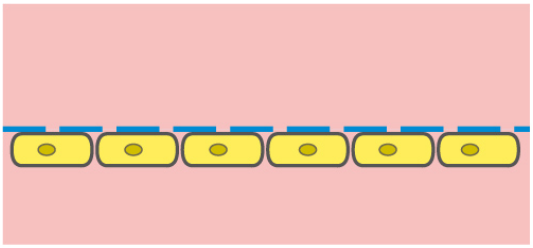
Applying liquid perfusion to the **upper** channel is not recommended. This is due to the different channel heights which create different shear stress conditions in the upper channel. Gas perfusion in the upper channel is possible.
Liquid flow and shear stress in the **lower** channel is homogeneous over the channel's and membrane's surface.

Applications

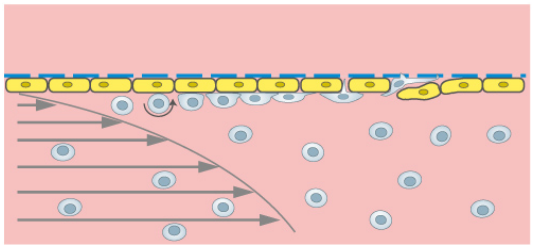
The µ–Slide Membrane ibiPore Flow can be perfused in the lower chamber for all flow applications. Detailed information about flow rates, shear stress, and shear rates is provided in Application Note 11 "Shear stress and shear rates". Suitable Tube Adapter Sets are also available (see page 7). They consist of a tubing (20 cm) with inner diameter of 1.6 mm and adapters for the connection between the ibidi µ–Slide (female Luer) and the tubing of the pump in use.

A variety of cell culture applications can be performed in the µ–Slide Membrane ibiPore Flow. A selection is shown in the following drafts:

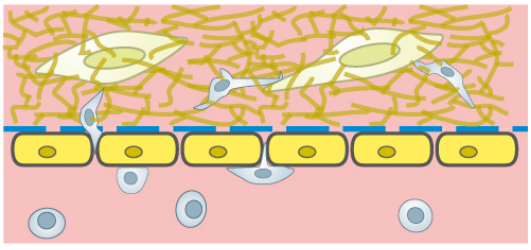
- Cultivation of a cell monolayer on one side of the membrane.



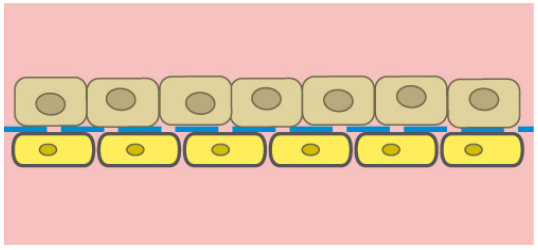
- Cultivation of a cell monolayer on one side of the membrane. Under flow conditions, rolling, adhesion, and transmigration of suspended leukocytes can be observed.



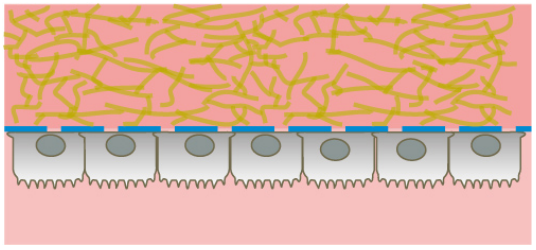
- Cultivation of a cell monolayer on one side of the membrane. Under flow conditions, rolling, adhesion, and transmigration of leukocytes towards cancer cells in a 3D matrix, producing chemoattractants, can be observed.



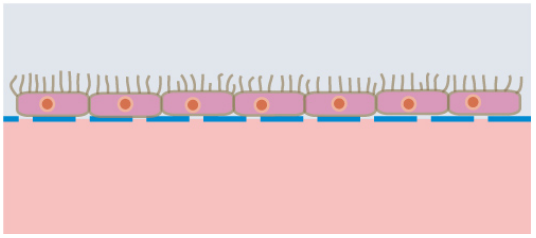
- Cultivation of separate cell monolayers on each side of the membrane. Signalling, co-culture, and transport studies are possible.



- Chemical factors inside of a 3D gel matrix lead to polarization of a cell monolayer which is cultured on the other side of the membrane.



- Polarized cell monolayer on one side of the membrane exposed to air flow (e.g. lung epithelial cells or skin models).



Cell culture under flow conditions

For perfusion of the lower channel, the shear stress (τ) can be calculated by inserting the flowrate (Φ) and the dynamical viscosity of the medium (η) in the following formula:

$$\tau \left[\frac{dyn}{cm^2}\right] = \eta \left[\frac{dyn \cdot s}{cm^2}\right] \cdot 131.6 \cdot \Phi \left[\frac{ml}{min}\right]$$

For simplicity, the calculation includes conversions of units (not shown).

Preparation for Cell Microscopy

To analyze your cells no special preparations are necessary. Cells can be observed live or fixed directly in the µ–Slide on an inverted microscope. You can use any fixative of your choice. The µ–Slide material is compatible with a

variety of chemicals, e.g. Acetone or Methanol. Further specifications can be found at www.ibidi.com. Due to the thin bottom of only 180 µm, high resolution microscopy is possible.

Important!

Due to the channel height of 0.4 mm the glass membrane can be imaged only with objective lenses with a working distance higher than 0.5 mm.

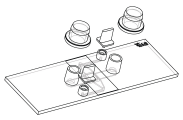
Immersion Oil

When using oil immersion objectives, use only the immersion oils specified in the table. The use of a non–recommended oil could lead to the damage of the plastic material and the objective.

Company	Product	Ordering Number
Zeiss	Immersol 518 F	(Zeiss) 444960
Zeiss	Immersol W 2010	(Zeiss) 444969
Leica	Immersion liquid	(Leica) 11513859

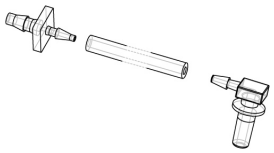
Ordering Information

µ–Slide Membrane ibiPore Flow The µ–Slide Membrane ibiPore Flow is available in different product versions.



Cat. No.	Description	Pcs./Box
85016	**µ–Slide Membrane ibiPore$^{0.5\,\mu m/20\%}$ Flow ibiTreat**: #1.5 polymer coverslip, tissue culture treated, 0.5 µm porous glass membrane, 20% porosity, sterilized	10
85026	**µ–Slide Membrane ibiPore$^{3.0\,\mu m/5\%}$ Flow ibiTreat**: #1.5 polymer coverslip, tissue culture treated, 3.0 µm porous glass membrane, 5% porosity, sterilized	10
85036	**µ–Slide Membrane ibiPore$^{5.0\,\mu m/5\%}$ Flow ibiTreat**: #1.5 polymer coverslip, tissue culture treated, 5.0 µm porous glass membrane, 5% porosity, sterilized	10
85046	**µ–Slide Membrane ibiPore$^{8.0\,\mu m/5\%}$ Flow ibiTreat**: #1.5 polymer coverslip, tissue culture treated, 8.0 µm porous glass membrane, 5% porosity, sterilized	10
85016-S	**µ–Slide Membrane ibiPore$^{0.5\,\mu m/20\%}$ Flow ibiTreat Trial Pack**: #1.5 polymer coverslip, tissue culture treated, 0.5 µm porous glass membrane, 20% porosity, sterilized	2
85026-S	**µ–Slide Membrane ibiPore$^{3.0\,\mu m/5\%}$ Flow ibiTreat Trial Pack**: #1.5 polymer coverslip, tissue culture treated, 3.0 µm porous glass membrane, 5% porosity, sterilized	2
85036-S	**µ–Slide Membrane ibiPore$^{5.0\,\mu m/5\%}$ Flow ibiTreat Trial Pack**: #1.5 polymer coverslip, tissue culture treated, 5.0 µm porous glass membrane, 5% porosity, sterilized	2
85046-S	**µ–Slide Membrane ibiPore$^{8.0\,\mu m/5\%}$ Flow ibiTreat Trial Pack**: #1.5 polymer coverslip, tissue culture treated, 8.0 µm porous glass membrane, 5% porosity, sterilized	2

Tube Adapter Set



Cat. No.	Description
10831	**Tube Adapter Set**: sterilized

In cooperation with SiMPore Inc.

For research use only!

Further technical specifications can be found at www.ibidi.com. For questions and suggestions please contact us by e-mail *info@ibidi.de* or by telephone +49 (0)89/520 4617 0. All products are developed and produced in Germany.