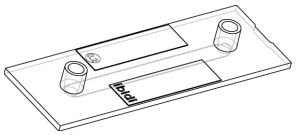


The ibidi product family is comprised of a variety of µ–Slides and µ–Dishes, which have all been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength.
The µ–Slides I Luer are designed for cell culture under perfusion and all flow applications. Main applications are the simulation of blood vessels for arteriosclerosis research and applying defined shear stress and shear rates on cells inside the channel. The female Luers allow easy connections to tubing and pump systems. The µ–Slide I Luer comes in five versions which only differ in their channels' heights and channel volumes.

Material

ibidi µ–Slides, µ–Dishes, and µ–Plates are made of a plastic that has the highest optical quality. The bottom material 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 plastic bottom, which should not be covered.

Optical Properties ibidi Standard Bottom	
Refractive index n_D (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 µm)
Material	microscopy plastic

Please note! The ibidi standard bottom is compatible with certain types of immersion oil only. A list of suitable oils can be found on page 4.

Geometry of the µ–Slides I Luer

The µ–Slides I Luer provide standard slide format according to ISO 8037/1.

General Dimensions	
Number of Channels	1
Channel length	50 mm
Channel width	5.0 mm
Volume per reservoir	60 µl
Growth area	2.5 cm^2 per channel
Bottom matches coverslip	No. 1.5

The channel volume differs, depending on the channel height (see table below).

Product name	Channel height	Channel volume
µ–Slide I $^{0.1}$ Luer	100 µm	25 µl
µ–Slide I $^{0.2}$ Luer	200 µm	50 µl
µ–Slide I $^{0.4}$ Luer	400 µm	100 µl
µ–Slide I $^{0.6}$ Luer	600 µm	150 µl
µ–Slide I $^{0.8}$ Luer	800 µm	200 µl

µ–Slide Surfaces

Depending on the type of cells and the special application you are using, you will need µ–Slides with different surfaces. If you do not require any special adhesion molecules for your application, the best choice will be ibiTreat, a tissue culture treated surface.

We provide precoated µ–Slides with adhesion substrates like Collagen IV, Fibronectin, Poly–L–Lysin, and Poly–D–Lysin. Such adhesion substrates have been shown to stimulate the adhesion and growth of various cell lines in µ–Slides. Only high–quality substrates are used [1].

The uncoated µ–Slide is manufactured from hydrophobic plastic. For the cultivation of most cell lines, it is indispensable to treat the uncoated µ–Slide with biopolymers, which mediate cell adhesion and growth.

Preparation for Cell Microscopy

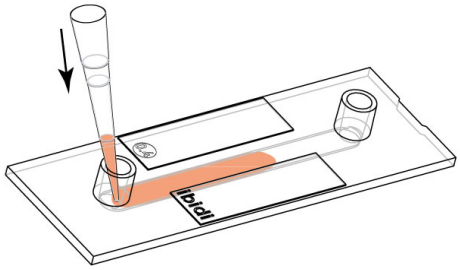
To analyze your cells, no special preparations are necessary. Cells can be observed live, or fixed directly in the µ–Slide on an inverted microscope. You can use any fixative of your choice. The µ–Slide material is compatible with a variety of chemicals (e.g., Acetone or Methanol). Further specifications can be found at www.ibidi.com. Due to the thin bottom of only 180 µm, high resolution microscopy is possible.

[1] Collagen IV: Corning #356233, Fibronectin: Corning #354008, Poly–L–Lysin: Sigma #P4832, Poly–D–Lysin: Corning #354210

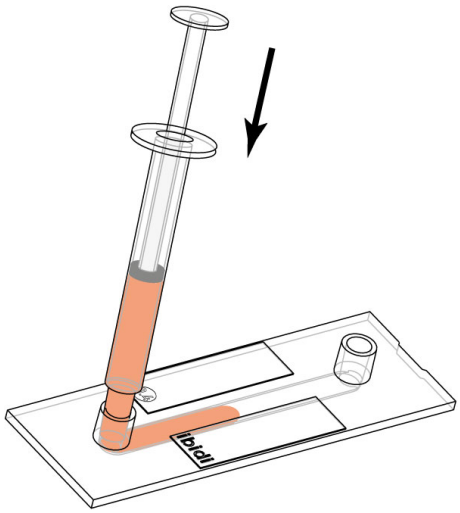
Filling and Handling of Channel Slides

In order to avoid air bubbles inside the channels please follow the recommendations below.

When filling the channels put the pipet tip directly to the channel's inlet. Apply the volume with a constant and swift flow.



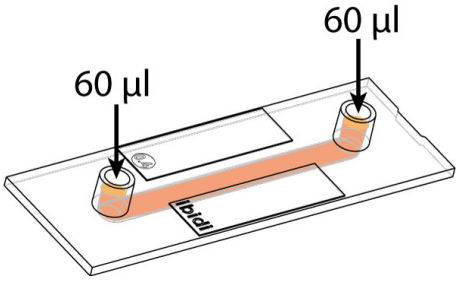
In special cases, e.g. when the channel surface is hydrophobic or when filling small channels, it might be necessary to fill the channel with a syringe. Use a low volume syringe with 1 or 2.5 ml!



Important!

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

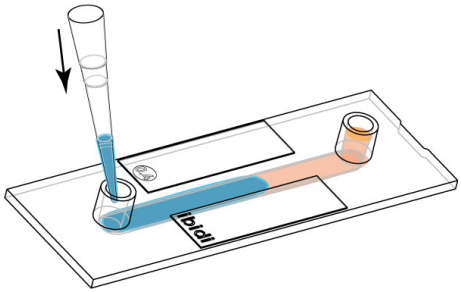
After cell attachment fill 60 µl in each well, for a better medium supply to the cells.



Medium Exchange

The following medium exchange protocol is important for cell culture medium exchange, staining, washing and coating procedures.

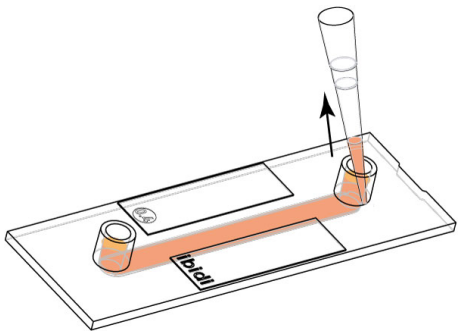
Empty the reservoirs completely without emptying the channel. Inject the new solution from one side and remove the old solution from the other side. Make sure the old solution is completely replaced. For a 99% exchange add about three times the channel volume from one side.



Important!

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

When aspirating the liquid put the pipet tip away from the channel's inlet! This prevents you from evacuating the whole channel.



Coating your µ–Slides I Luer

The uncoated µ–Slide must be coated to promote cell adhesion. If you want to establish a certain coating to match your needs, we recommend testing your coating procedure on both uncoated and ibiTreat µ–Slides, since we have observed that some biomolecules adhere differently to hydrophobic and hydrophilic plastic surfaces.

- Prepare your coating solution according to the manufacturer's specifications or reference.
- Apply the channel volume depending on the channel height. Leave at room temperature for at least 30 minutes.

Product name	Coating area	Growth area
µ–Slide I $^{0.1}$ Luer	5.1 cm^2	2.5 cm^2
µ–Slide I $^{0.2}$ Luer	5.2 cm^2	2.5 cm^2
µ–Slide I $^{0.4}$ Luer	5.4 cm^2	2.5 cm^2
µ–Slide I $^{0.6}$ Luer	5.6 cm^2	2.5 cm^2
µ–Slide I $^{0.8}$ Luer	5.8 cm^2	2.5 cm^2

- Aspirate the solution and wash with the recommended protein dilution buffer. You can add the buffer into one channel end and simultaneously aspirate it on the other side.
- Optionally let dry at room temperature. Attention, some coating proteins might degenerate when drying!

Further information about coatings are provided in Application Note 08 "Cell culture coating".

Cell Culture under Static Conditions

For many static applications with microscopic imaging, like transfection, immunofluorescence staining or cell morphology the µ–Slide I Luer is an optimal solution.

Important!

The µ–Slide I $^{0.1}$ Luer and µ–Slide I $^{0.2}$ Luer are not recommended for use in static cell culture!
For longer cultivation, a gentle flow is necessary. This can be achieved by a perfusion system or an incubator-compatible cell culture rocker.

- Trypsinize and count cells as usual. The cell density after seeding strongly depends on the channel's height. We recommend the following cell concentrations and volumes:

Product name	Volume	Cell concentration
µ–Slide I $^{0.1}$ Luer	25 µl	12–28 x 10^5 cells/ml
µ–Slide I $^{0.2}$ Luer	50 µl	6–14 x 10^5 cells/ml
µ–Slide I $^{0.4}$ Luer	100 µl	3–7 x 10^5 cells/ml
µ–Slide I $^{0.6}$ Luer	150 µl	2–4.5 x 10^5 cells/ml
µ–Slide I $^{0.8}$ Luer	200 µl	1.5–3.5 x 10^5 cells/ml

- Apply the volume directly into the channel. The recommended cell concentration should result in a 50 % optical confluence layer after 24 hours.
- Cover reservoirs with the supplied caps. Incubate at 37°C and 5 % CO_2 as usual.
- After cell attachment fill each reservoir with 60 µl medium.

Depending on the cells we recommend exchanging the medium every day in static culture: Aspirate both reservoirs (not the channel). Flush fresh medium inside the channel by filling one reservoir with 120 µl medium and removing the content of the reservoir from the other well, ensuring the channel is never dry. Leave both reservoirs filled with approx. 60 µl each.

Tip:

The day before seeding the cells we recommend placing the cell medium, the µ–Slide, and the tubing into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles over the incubation time.
Quick dispensing of cell suspension helps to avoid trapped air bubbles and leads to maximal homogeneity of cell distribution.

Cell Culture under Flow Conditions

Due to the Luer adapters, µ–Slide I Luer is suitable to any fluidic setup for cell cultivation under flow conditions. Cells are seeded into the channel and the flow is applied after cell attachment.

- Trypsinize and count cells as usual. The cell density after seeding strongly depends on the channel's height. We recommend the following cell concentrations and volumes:

Product name	Volume	Cell concentration
µ–Slide I $^{0.1}$ Luer	25 µl	5–10 x 10^6 cells/ml
µ–Slide I $^{0.2}$ Luer	50 µl	2.5–5 x 10^6 cells/ml
µ–Slide I $^{0.4}$ Luer	100 µl	1.2–2.5 x 10^6 cells/ml
µ–Slide I $^{0.6}$ Luer	150 µl	0.8–1.6 x 10^6 cells/ml
µ–Slide I $^{0.8}$ Luer	200 µl	0.6–1.2 x 10^6 cells/ml

- Apply the volume directly into the channel. The recommended cell concentration should result in a 100 % optical confluence layer after some hours.
- Cover reservoirs with the supplied caps. Incubate at 37°C and 5 % CO_2 as usual.
- After cell attachment fill each reservoir with 60 µl medium.
- The µ–Slide is now ready for applying flow conditions on the adherent cells. Don't trap air bubbles when plugging in the connecting tubes.

Tip:

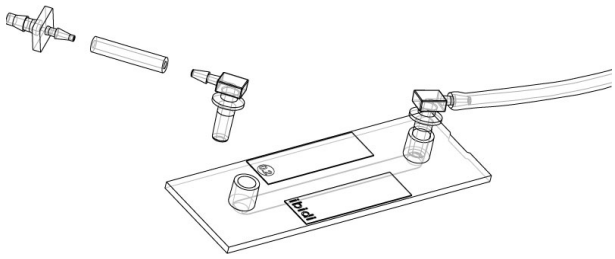
The day before seeding the cells we recommend placing the cell medium, the µ–Slide, and the tubing into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles over the incubation time.
Quick dispensing of cell suspension helps to avoid trapped air bubbles and leads to maximal homogeneity of cell distribution.
For long term analysis of cells under flow conditions we recommend using µ–Slides with ibiTreat surface.

Application Note 13 "HUVECs under perfusion" describes a detailed protocol of a long term experiment with HUVECs and the ibidi Pump System.

Detailed information about flow rates, shear stress, and shear rates is provided in Application Note 11 "Shear stress and shear rates" on www.ibidi.com.

For connecting several µ–Slides I Luer with each other in a serial way, please refer to our Application Note 25 "Serial Connection of Flow Chamber".

Suitable Tube Adapter Sets are also available (see page 4). 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.



Please contact us for recommended perfusion setups. ibidi provides a variety of channel slides and pump systems.

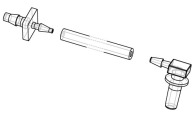
Immersion Oil

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
ibidi	Immersion Oil	(ibidi) 50101
Zeiss	Immersol 518 F	(Zeiss) 444960
Zeiss	Immersol W 2010	(Zeiss) 444969
Leica	Immersion liquid	(Leica) 11513859

Tube Adapter Set

For the connection of the ibidi µ–Slides to any flow system suitable Tube Adapter Sets are also available. 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.



Ordering Number	Product Name	Characteristics
10831	Tube Adapter Set	12 pcs, sterile

µ–Slide I Luer Family

The µ–Slide I Luer family is available with different channel heights and surfaces. See table below for choosing your µ–Slide I Luer.

µ–Slide I $^{0.1}$ Luer

Ordering Number	Treatment or Coating	Characteristics
81122	Collagen IV, sterile	protein coating
81123	Fibronectin, sterile*	protein coating
81121	uncoated, sterile	hydrophobic

µ–Slide I $^{0.2}$ Luer

Ordering Number	Treatment or Coating	Characteristics
80166	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
80162	Collagen IV, sterile	protein coating
80163	Fibronectin, sterile*	protein coating
80164	Poly-L-Lysine, sterile	biopolymer coating
80165	Poly-D-Lysine, sterile*	biopolymer coating
80161	uncoated, sterile	hydrophobic

µ–Slide I $^{0.4}$ Luer

Ordering Number	Treatment or Coating	Characteristics
80176	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
80172	Collagen IV, sterile	protein coating
80173	Fibronectin, sterile*	protein coating
80174	Poly-L-Lysine, sterile	biopolymer coating
80175	Poly-D-Lysine, sterile*	biopolymer coating
80171	uncoated, sterile	hydrophobic

µ–Slide I $^{0.6}$ Luer

Ordering Number	Treatment or Coating	Characteristics
80186	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
80182	Collagen IV, sterile	protein coating
80183	Fibronectin, sterile*	protein coating
80184	Poly-L-Lysine, sterile	biopolymer coating
80185	Poly-D-Lysine, sterile*	biopolymer coating
80181	uncoated, sterile	hydrophobic

µ–Slide I $^{0.8}$ Luer

Ordering Number	Treatment or Coating	Characteristics
80196	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
80192	Collagen IV, sterile	protein coating
80193	Fibronectin, sterile*	protein coating
80194	Poly-L-Lysine, sterile	biopolymer coating
80195	Poly-D-Lysine, sterile*	biopolymer coating
80191	uncoated, sterile	hydrophobic

* available on request only

Selected References

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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.