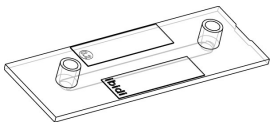


The ibidi product family comprises a variety of different shapes of µ–Slides and µ–Dishes which all have been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments 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 and µ–Dishes consist of a plastic with highest optical quality. The material exhibits extremely low birefringence and autofluorescence, both similar to that of glass. It is not possible to detach the bottom from the upper part. The µ–Slides and µ–Dishes are not autoclavable since they are temperature stable up to 80°C/175°F only. 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

Geometry of the µ-Slides I Luer

The µ–Slides I Luer provide standard slide format according to ISO 8037/1. The bottom matches coverslip No. 1.5.

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 your cells and special application you will need µ–Slides with different surfaces. If you do not need 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 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 cultivation of most cell lines it is indispensable to treat the uncoated µ–Slide with biopolymers which mediate cell adhesion and growth.

Coating your µ-Slides I Luer

The uncoated µ-Slide must be coated to promote cell adhesion. If you like to establish a certain coating for your demands we recommend to test your coating procedure on uncoated and ibiTreat µ-Slides, since we have observed that some biomolecules adhere differently to hydrophobic or 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 1 ml ultra-pure water. Let dry at room temperature.

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

[1] Collagen IV, BD Cat.–Nr. 35 6233, Fibronectin, BD Cat.–Nr. 354008, Poly–L–Lysin, Sigma Cat.–Nr. P4832, Poly–D–Lysin, BD Cat.–Nr. 354210

Seeding cells

Important!

The µ–Slide I $^{0.1}$ Luer and µ–Slide I $^{0.2}$ Luer are not recommended for use in static cell culture!

- 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	24 x 10^5 cells/ml
µ–Slide I $^{0.2}$ Luer	50 µl	12 x 10^5 cells/ml
µ–Slide I $^{0.4}$ Luer	100 µl	6 x 10^5 cells/ml
µ–Slide I $^{0.6}$ Luer	150 µl	4 x 10^5 cells/ml
µ–Slide I $^{0.8}$ Luer	200 µl	3 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 24h.
- 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.

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.

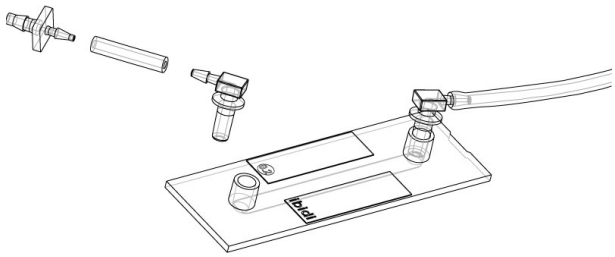
Tip:

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.
For long term analysis of cells under flow conditions we recommend using µ–Slides with ibiTreat as surface.

Flow application

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.

Suitable flow kits (µ-Slide I Luer + tubing and adapters) are also available.



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

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.

Immersion oil

When using oil immersion objectives, only the immersion oils specified in the table may be used. The use of different oil can lead to damages of the plastic material and the objective.

Company	Product	Ordering number
Cargille	type DF, Formula Code: 1261	(Cargille) 16242
Zeiss	518 F	(Zeiss) 444960
Olympus	50CC	(Olympus) 35506
Nikon	50 CCM DF	(Nikon) MXA 20351
Leica	immersion oil, low fluorescence	(Leica) 11513859

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
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
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
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
80191	uncoated, sterile	hydrophobic

* available on request only

Selected cell tests on different surfaces

Many eukaryotic and bacterial cells have been tested by ibidi on the different surfaces of the µ-Slides. A variety of other cell lines like COS, CHO, HepG2, and NIH 3T3 were successfully grown by our customers.

	ibiTreat	Collagen IV	Fibronectin	Poly-L-Lysin	Poly-D-Lysin	uncoated
HUVEC	excellent	good	excellent	no cell growth	not done	no cell growth
Rat1	excellent	excellent	excellent	excellent	excellent	poor
HT1080	excellent	excellent	excellent	excellent	not done	poor
HeLa	excellent	excellent	excellent	excellent	not done	poor
Neuro2A	excellent	excellent	excellent	excellent	excellent	poor
PC12	good	excellent	excellent	excellent	excellent	no cell growth
Dictyostelium discoideum	not done	excellent	not done	not done	not done	excellent
Escherichia coli	excellent	not done	not done	excellent	not done	excellent

HUVEC = Human Umbilical Vein Endothelial Cells
Rat1 = Rat Fibroblast
HT1080 = Human Fibrosarcoma
HeLa = Human Cervix Adenocarcinoma
Neuro2A = Mouse Neuroblastoma
PC12 = Rat Pheochromocytom

Dictyostelium discoideum = strain wild type AX-2

Escherichia coli = strain MDG131

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.