

The ibidi product family comprises a variety of different shapes of μ-Slides, μ-Dishes and plates 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-Slide y-shaped can be easily connected to a pump via its Luer connectors. It enables you to grow cells under flow conditions with a bifurcation of 30 and 45 degree depending on the flow direction you choose. It is

meant as a simulation system for blood vessels where the reaction of cells to a stimulus of your choice can be observed in real-time.

Material

The μ-Slides 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 slide. The μ-Slides are not autoclavable since they are temperature stable up to 60°C/140°F only. Please note that gas exchange between the channel and incubator's atmosphere occurs partially through the plastic bottom which should not be covered. Thus, it is recommended to place the μ-Slide on an ibidi μ-Slide rack which can be purchased from your local distributor.

Geometry of the μ-Slide y-shaped

The μ-Slide y-shaped provides standard slide format according to ISO 8037/1.

Dimensions	
Channel volume	110 μl
Channel height	0.4 mm
Branching angles	30° and 45°
Adapters	female Luer
Volume per reservoir	60 μl
Growth area	2.8 cm ²
Coating area using 110 μl	5.6 cm ²
Bottom matches coverslip	No. 1.5

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.

¹Collagen 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

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-Slide y-shaped

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 110 μl of the solution as it is shown in the movie manuals on www.ibidi.com. Leave at room temperature for at least 30 minutes.
- 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.

Seeding cells

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a $3-7 \times 10^5$ cells/ml suspension should result in a confluent layer within 2-3 days.
- Apply 200 μl cell suspension into the channel of the μ-Slide using a 1000 μl pipette.
- Remove the cell suspension from the reservoirs.
- Cover reservoirs with the supplied caps. Incubate at 37°C and 5% CO₂ as usual.

Instructions

μ-Slide y-shaped

- After cell attachment refill each reservoir with 60 μl medium for longer cultivation.
- 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 to exchange the medium every day in static culture: Aspirate both reservoirs. Flush fresh medium inside the channel by filling one reservoir with 400 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 to place 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.

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 to use μ-Slides with ibiTreat as surface.

Preparation for cell microscopy

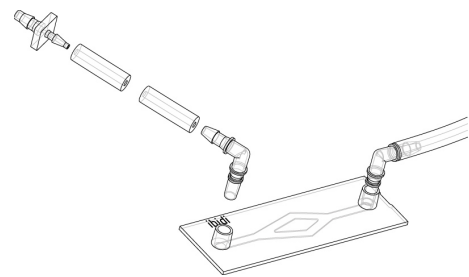
To analyze your cells no special preparations are necessary. Cells can be observed live or fixed directly in the μ-Slide on an inverted microscope. You can use any fixative of your choice. The μ-Slide material is compatible with a variety of chemicals, e.g. Acetone or Methanol. Further specifications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

bottom of only 180 μm, high resolution microscopy is possible.

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 y-shaped + tubing and adapters) are also available.



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

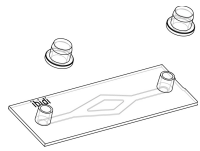
Immersion oil

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 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 y-shaped family

The μ-Slide y-shaped family is available with different surfaces. See table below for choosing your μ-Slide y-shaped.

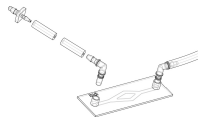


Ordering number	Treatment or Coating	characteristics
80126	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
80122	Collagen IV, sterile	protein coating
80123	Fibronectin, sterile*	protein coating
80124	Poly-L-Lysine, sterile	biopolymer coating
80125	Poly-D-Lysine, sterile*	biopolymer coating
80121	uncoated, sterile	hydrophobic

* available on request only

μ-Slide y-shaped flow kits

The μ-Slide y-shaped is available as flow kit with tubes and adapters.



Ordering number	Treatment or Coating	characteristics
80146	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
80144	Collagen IV, sterile	protein coating
80145	uncoated, sterile	hydrophobic

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, HepG3, 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
<i>Dictyostelium discoideum</i>	not done	excellent	not done	not done	not done	excellent
<i>Escherichia coli</i>	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.

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