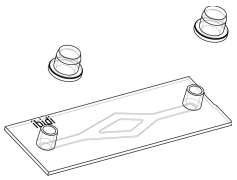


The ibidi product family is comprised of a variety of µ–Slides and µ–Dishes, which have all been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised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

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 2.**

## 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.

## Geometry of the µ–Slide y–shaped

The µ–Slide y–shaped provides a 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 |

## Coating your µ-Slide y-shaped

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 200 µl of the coating solution into the channel of the µ–Slide using a 1000 µl pipette.
- Remove 90 µl coating solution from the reservoirs.
- Leave at room temperature for at least 30 minutes.
- Aspirate the solution and wash with the recommended protein dilution buffer. You can add the

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

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.

## 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 × $10^5$ 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 % $CO_2$ as usual.
- After cell attachment fill 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 slowly 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 the ibiTreat surface.

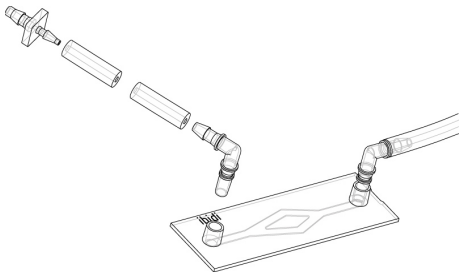
## Preparation for Cell Microscopy

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" and Application Note 18 "Shear Stress and Shear Rates in µ–Slide y–shaped".

Suitable Tube Adapter Sets are also available (see page 3). 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.

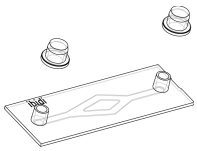
## Immersion Oil

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 |

## 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.

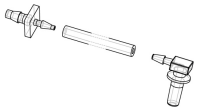


| Ordering Number | Treatment or Coating | Characteristics |
|---|---|---|
| 80126 | ibiTreat, tissue culture treated, sterile | hydrophilic, tissue culture treated |
| 80122 | Collagen IV, sterile | protein coating |
| 80123 | Fibronectin, sterile* | protein coating |
| 80124 | Poly-L-Lysine, sterile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

* available on request only

## 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 |

## Selected References

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J. Samarin, I. Cicha, and M. Goppelt-Struebe. Cell type–specific regulation of CCN2 protein expression by PI3K–AKT–FoxO signaling. *Journal of Cell Communication and Signaling*, 2009. doi: 10.1007/s12079-009-0055-5.

M. J. Seidler, S. Salvenmoser, and F.-M. C. Muller. Aspergillus fumigatus Forms Biofilms with Reduced Antifungal Drug Susceptibility on Bronchial Epithelia Cells. *Antimicrob. Agents Chemother.*, 2008. doi: 10.1128/aac.00234-08.

K. Urschel, C. D. Garlichs, W. G. Daniel, and I. Cicha. VEGFR2 signalling contributes to increased endothelial susceptibility to TNF–$\alpha$ under chronic non–uniform shear stress. *Atherosclerosis*, 2011.

## 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.